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PROCESSING COMPLETED FOR L1
L2 10 DUP REM L1 (0 DUPLICATES REMOVED)

=> d l2 1-10 bib ab

L2 ANSWER 1 OF 10 USPATFULL
AN 2002:54665 USPATFULL
TI Glucan-containing compositions and paper
IN Nichols, Scott E., Johnston, IA, UNITED STATES
PI US 2002031826 A1 20020314
AI US 2000-740274 A1 20001219 (9)
RLI Division of Ser. No. US 1998-210361, filed on 11 Dec 1998, PENDING Continuation-in-part of Ser. No. US 1998-9620, filed on 20 Jan 1998, GRANTED, Pat. No. US 6127603 Continuation-in-part of Ser. No. US 1998-7999, filed on 16 Jan 1998, GRANTED, Pat. No. US 6087559 Continuation-in-part of Ser. No. US 1998-8172, filed on 16 Jan 1998, GRANTED, Pat. No. US 6127602 Continuation of Ser. No. US 1995-485243, filed on 7 Jun 1995, GRANTED, Pat. No. US 5712107 Continuation of Ser. No. US 1995-478704, filed on 7 Jun 1995, ABANDONED Continuation of Ser. No. US 1995-482711, filed on 7 Jun 1995, ABANDONED
DT Utility
FS APPLICATION
LREP Catherine D. Brooke, Patent Agent, 7100 N.W. 62nd Avenue, P.O. Box 1000, Johnston, IA, 50131-1000
CLMN Number of Claims: 34
ECL Exemplary Claim: 15
DRWN No Drawings
LN.CNT 3136
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides methods of making paper, utilizing glucans, produced by the glucosyltransferase B, C or D enzyme of the species Streptococcus mutans, instead of modified starches. The present glucans are functionally similar to currently utilized modified starches and are particularly useful in the coating step of paper manufacture. The present glucans also exhibit thermoplastic properties and impart gloss to the paper during the coating step.

L2 ANSWER 2 OF 10 USPATFULL
AN 2001:237672 USPATFULL
TI Recombinant bacterial phytases and uses thereof
IN Short, Jay M., Rancho Santa Fe, CA, United States
Kretz, Keith A., San Marcos, CA, United States
PA Diversa Corporation (U.S. corporation)
PI US 2001055788 A1 20011227
AI US 2001-777566 A1 20010205 (9)
RLI Continuation of Ser. No. US 1999-318528, filed on 25 May 1999, GRANTED, Pat. No. US 6183740 Continuation-in-part of Ser. No. US 1999-291931, filed on 13 Apr 1999, GRANTED, Pat. No. US 6190897 Continuation of Ser. No. US 1999-259214, filed on 1 Mar 1999, GRANTED, Pat. No. US 6110719 Division of Ser. No. US 1997-910798, filed on 13 Aug 1997, GRANTED, Pat. No. US 5876997
DT Utility
FS APPLICATION

LREP Lisa A. Haile, Ph.D., Gray Cary Ware & Freidenrich LLP, Suite 1600, 4365 Executive Drive, San Diego, CA, 92121-2189
CLMN Number of Claims: 15
ECL Exemplary Claim: 1
DRWN 4 Drawing Page(s)
LN.CNT 2934
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A purified recombinant phytase enzyme derived from Escherichia coli B. The enzyme has a molecular weight of about 47.1 kilodaltons and has phytase activity (SEQ ID NO:2). The enzyme can be produced from native or recombinant host cells and can be used to aid in the digestion of phytate where desired. In particular, the phytase of the present invention can be used in foodstuffs to improve the feeding value of phytate rich ingredients.

L2 ANSWER 3 OF 10 USPATFULL
AN 2001:197264 USPATFULL
TI Maize aquaporins and uses thereof
IN Jung, Rudolf, Des Moines, IA, United States
Chaumont, Francois, Louvain-la-Neuve, Belgium
Chrispeels, Maarten, La Jolla, CA, United States
PA Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S. corporation)
The Regents of the University of California, Oakland, CA, United States (U.S. corporation)
PI US 6313376 B1 20011106
AI US 1999-372448 19990811 (9)
PRAI US 1998-96627P 19980814 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Fox, David T.; Assistant Examiner: Ibrahim, Medina A.
LREP Pioneer Hi-Bred International, Inc.
CLMN Number of Claims: 40
ECL Exemplary Claim: 1,4,5,8,13
DRWN No Drawings
LN.CNT 3369
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides isolated maize aquaporin nucleic acids and their encoded proteins. The present invention provides methods and compositions relating to altering aquaporin concentration and/or composition of plants. The invention further provides recombinant expression cassettes, host cells, transgenic plants, and antibody compositions.

L2 ANSWER 4 OF 10 USPATFULL
AN 2001:197263 USPATFULL
TI Maize aquaporins and uses thereof
IN Jung, Rudolf, Des Moines, IA, United States
Barrieu, Francois, Bordeaux, France
PA Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S. corporation)
PI US 6313375 B1 20011106
AI US 1999-372422 19990811 (9)
PRAI US 1998-98692P 19980813 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Fox, David T.; Assistant Examiner: Ibrahim, Medina A.

LREP Pioneer Hi-Bred International, Inc.
CLMN Number of Claims: 40
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 3234
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides isolated maize aquaporin nucleic acids and their encoded proteins. The present invention provides methods and compositions relating to altering aquaporin concentration and/or composition of plants. The invention further provides recombinant expression cassettes, host cells, transgenic plants, and antibody compositions.

L2 ANSWER 5 OF 10 USPATFULL
AN 2001:185451 USPATFULL
TI Intracellular antifreeze polypeptides and nucleic acids
IN Hew, Choy, Thornhill, Canada
Gong, Zhiyuan, Toronto, Canada
PA HSC Research and Development Ltd. Partnership, Toronto, Canada (non-U.S. corporation)
PI US 6307020 B1 20011023
WO 9728260 19970807
AI US 1998-117121 19981120 (9)
WO 1997-CA62 19970130
19981120 PCT 371 date
19981120 PCT 102(e) date
DT Utility
FS GRANTED
EXNAM Primary Examiner: Carlson, Karen Cochrane; Assistant Examiner: Robinson, Hope A.
LREP Townsend and Townsend and Crew LLP
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN 21 Drawing Figure(s); 20 Drawing Page(s)
LN.CNT 2175
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A family of related intracellular skin type antifreeze polypeptides and corresponding coding nucleic acids are provided. These are the first skin type intracellular antifreeze polypeptides and coding nucleic acids ever reported. The polypeptides are naturally expressed in the skin of Winter Flounder, and skin specific promoters are also provided. The polypeptides are used to make cells cold-resistant, and to improve the palatability of cold foods and liquids. Cold resistant eukaryotes and prokaryotes, including plants, animals and bacteria are made using the skin-type intracellular antifreeze polypeptides and nucleic acids.

L2 ANSWER 6 OF 10 USPATFULL
AN 2001:147690 USPATFULL
TI Substitutes for modified starch and latexes in paper manufacture
IN Nichols, Scott E., Johnston, IA, United States
PA Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S. corporation)
PI US 6284479 B1 20010904
AI US 1998-210361 19981211 (9)
RLI Continuation-in-part of Ser. No. US 1998-8172, filed on 16 Jan 1998
Division of Ser. No. US 1995-482711, filed on 7 Jun 1995, now abandoned
Continuation-in-part of Ser. No. US 1998-9620, filed on 20 Jan 1998

Continuation of Ser. No. US 1995-485243, filed on 7 Jun 1995, now patented, Pat. No. US 5712107 Continuation-in-part of Ser. No. US 1998-7999, filed on 16 Jan 1998 Division of Ser. No. US 1995-478704, filed on 7 Jun 1995, now abandoned
DT Utility
FS GRANTED
EXNAM Primary Examiner: Leary, Louise N.
LREP Pioneer Hi-Bred International, Inc.
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN 5 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 1789
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides methods of making paper, utilizing glucans, produced by the glucosyltransferase B, C or D enzyme of the species Streptococcus mutans, instead of modified starches. The present glucans are functionally similar to currently utilized modified starches and are particularly useful in the coating step of paper manufacture. The present glucans also exhibit thermoplastic properties and impart gloss to the paper during the coating step.

L2 ANSWER 7 OF 10 USPATFULL
AN 2001:117241 USPATFULL
TI Pyruvate dehydrogenase kinase polynucleotides, polypeptides and uses thereof
IN Randall, Douglas D., Columbia, MO, United States
Thelen, Jay J., Columbia, MO, United States
Miernyk, Jan A., Peoria, IL, United States
Muszynski, Michael G., Des Moines, IA, United States
Sewalt, Vincent J. H., West Des Moines, IA, United States
PA Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S. corporation)
University of Missouri, Columbia, MO, United States (U.S. corporation)
PI US 6265636 B1 20010724
AI US 1999-333423 19990615 (9)
PRAI US 1998-89998P 19980619 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Fox, David T.; Assistant Examiner: Ibrahim, Medina A.
LREP Pioneer Hi-Bred International, Inc.
CLMN Number of Claims: 52
ECL Exemplary Claim: 12,19
DRWN No Drawings
LN.CNT 3517
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides methods and compositions relating to altering carbohydrate metabolism and/or composition of plants. The invention provides isolated nucleic acids and their encoded proteins, expression cassettes, host cells, transgenic plants, and antibody compositions.

L2 ANSWER 8 OF 10 USPATFULL
AN 2001:48312 USPATFULL
TI Hm2 cDNA from maize encoding disease resistance polypeptide
IN Briggs, Steven P., DelMar, CA, United States
Johal, Gurmukh, Columbia, MO, United States
Multani, Dilbag Singh, Columbia, MO, United States
PA Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S.

corporation)
The Curators of the University of Missouri, Columbia, MO, United States (U.S. corporation)
PI US 6211440 B1 20010403
AI US 1999-231227 19990114 (9)
PRAI US 1998-71684P 19980116 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Nelson, Amy J.
LREP Pioneer Hi-Bred International, Inc.
CLMN Number of Claims: 14
ECL Exemplary Claim: 2
DRWN No Drawings
LN.CNT 3025
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides isolated Hm2 nucleic acids. The invention further provides expression cassettes, transferred host cells, and transgenic plants. Also, the invention provides methods of imparting disease resistance to plants susceptible to fungal pathogens, which utilize cyclic tetrapeptide toxins.

L2 ANSWER 9 OF 10 USPATFULL
AN 2001:29788 USPATFULL
TI Alteration of hemicellulose concentration in plants
IN Dhugga, Kanwarpal S., Johnston, IA, United States
Nichols, Scott E., Johnston, IA, United States
Fallis, Patricia Lynne, Polk City, IA, United States
PA Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S. corporation)
PI US 6194638 B1 20010227
AI US 1999-338671 19990622 (9)
PRAI US 1998-90416P 19980623 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Fox, David T.; Assistant Examiner: Ibrahim, Medina A
LREP Pioneer Hi-Bred International, Inc.
CLMN Number of Claims: 20
ECL Exemplary Claim: 1,11
DRWN No Drawings
LN.CNT 3616
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention provides isolated Rgp nucleic acids and their encoded proteins. The present invention provides methods and compositions relating to altering RGP levels in plants. The invention further provides recombinant expression cassettes, host cells, transgenic plants, and antibody compositions.

L2 ANSWER 10 OF 10 USPATFULL
AN 2001:17988 USPATFULL
TI Recombinant bacterial phytases and uses thereof
IN Short, Jay M., Rancho Santa Fe, CA, United States
Kretz, Keith A., San Marcos, CA, United States
PA Diversa Corporation, San Diego, CA, United States (U.S. corporation)
PI US 6183740 B1 20010206
AI US 1999-318528 19990525 (9)
RLI Continuation-in-part of Ser. No. US 1999-291931, filed on 13 Apr 1999
Continuation of Ser. No. US 1999-259214, filed on 1 Mar 1999, now

patented, Pat. No. US 6110719 Division of Ser. No. US 1997-910798, filed on 13 Aug 1997, now patented, Pat. No. US 5876997
DT Utility
FS Granted
EXNAM Primary Examiner: Achutamurthy, Ponnathapu; Assistant Examiner: Tung, Peter
LREP Gray Cary Ware & Freidenrich LLP, Haile, Lisa A.
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 6 Drawing Figure(s); 5 Drawing Page(s)
LN.CNT 2800
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A purified recombinant phytase enzyme derived from Escherichia coli B. The enzyme has a molecular weight of about 47.1 kilodaltons and has phytase activity (SEQ ID NO:2). The enzyme can be produced from native or recombinant host cells and can be used to aid in the digestion of phytate where desired. In particular, the phytase of the present invention can be used in foodstuffs to improve the feeding value of phytate rich ingredients.

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COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	21.44	23.12

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=> s vaccine and (self-antigen)
L3 210 VACCINE AND (SELF-ANTIGEN)

=> s l3 and (transformed cells)
L5 18 L3 AND (TRANSFORMED CELLS)

=> s l3 and (tranfected cell!)

L6 0 L3 AND (TRANFECTED CELL!)

=> s l3 and (transfected cell!)
L7 19 L3 AND (TRANSFECTED CELL!)

=> s l5 or l7
L8 34 L5 OR L7

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PROCESSING COMPLETED FOR L8
L9 34 DUP REM L8 (0 DUPLICATES REMOVED)

=> d l9 1-34 bib ab

L9 ANSWER 1 OF 34 USPATFULL
AN 2002:67349 USPATFULL
TI Coupling of peripheral tolerance to endogenous IL-10 promotes effective modulation of T cells and ameliorates autoimmune disease
IN Zaghouani, Habib, Columbia, MO, UNITED STATES
PI US 2002038002 A1 20020328
AI US 2001-873901 A1 20010604 (9)
PRAI US 2000-209527P 20000605 (60)
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 620 NEWPORT CENTER DRIVE, SIXTEENTH FLOOR, NEWPORT BEACH, CA, 92660
CLMN Number of Claims: 65
ECL Exemplary Claim: 1
DRWN 45 Drawing Page(s)
LN.CNT 4140
AB Immunomodulating agents comprising at least one Fc receptor ligand and at least one immunosuppressive factor are provided as are methods for their manufacture and use. The immunomodulating agents may be in the form of polypeptides or chimeric antibodies and preferably incorporate an immunosuppressive factor comprising a T cell receptor agonist or antagonist. The compounds and compositions of the invention may be used to selectively suppress the immune system to treat symptoms associated with immune disorders such as allergies, transplanted tissue rejection and autoimmune disorders including autoimmune diabetes, rheumatoid arthritis and multiple sclerosis.

L9 ANSWER 2 OF 34 USPATFULL
AN 2002:16589 USPATFULL
TI Presentation of hydrophobic antigens to T-cells by CD1 molecules
IN Porcelli, Steven A., Bronx, NY, UNITED STATES
Brenner, Michael B., Newton, MA, UNITED STATES
Beckman, Evan M., Sudbury, MA, UNITED STATES
Furlong, Stephen T., Wilmington, DE, UNITED STATES
PI US 2002009465 A1 20020124
AI US 2001-861963 A1 20010521 (9)
RLI Division of Ser. No. US 1995-501600, filed on 12 Jul 1995, GRANTED, Pat. No. US 6238676 Continuation-in-part of Ser. No. US 1994-322980, filed on 13 Oct 1994, GRANTED, Pat. No. US 5679347 Continuation-in-part of Ser. No. WO 1994-US6991, filed on 21 Jun 1994, UNKNOWN Continuation-in-part of Ser. No. US 1993-80072, filed on 21 Jun 1993, ABANDONED Continuation-in-part of Ser. No. US 1992-989790, filed on 10 Dec 1992, UNKNOWN

DT Utility
FS APPLICATION
LREP Elizabeth R. Plumer, Wolf, Greenfield & Sacks, P.C., Federal Reserve Plaza, 600 Atlantic Avenue, Boston, MA, 02210
CLMN Number of Claims: 34
ECL Exemplary Claim: 1
DRWN 36 Drawing Page(s)
LN.CNT 2548
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Provided are CD-1 presented antigens, compositions, cells, inhibitors and methods relating to the use of hydrophobic antigen presentation by CD1 molecules, including:

methods for detecting the presence of a CD1-presented hydrophobic antigen in a sample;

methods for isolating such CD1-presented antigens and the isolated antigens;

vaccines containing CD1-presented antigens and vaccination methods;

methods of blocking CD1 antigen presentation;

methods of identifying and/or isolating CD1 blocking agents and the isolated CD1 blocking agents;

methods of inducing CD1 expression; and

T-cells for use in the methods disclosed herein.

L9 ANSWER 3 OF 34 USPATFULL
AN 2002:69599 USPATFULL
TI [redacted]
IN Halpern, Michael S., West Newton, MA, United States
England, James M, Media, PA, United States
PA Philadelphia Health and Educational Corporation, Philadelphia, PA, United States (U.S. corporation)
PI US 6365151 B1 20020402
AI US 1998-167322 19981007 (9)
RLI Continuation-in-part of Ser. No. US 1998-101226, filed on 2 Jul 1998, now abandoned Continuation-in-part of Ser. No. WO 1997-US582, filed on 13 Jan 1997
PRAI US 1996-10262P 19960119 (60)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Hauda, Karen M.; Assistant Examiner: Beckerleg, Anne-Marie S
LREP Drinker Biddle & Keath LLP
CLMN Number of Claims: 22
ECL Exemplary Claim: 1
DRWN 7 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 1838
AB [redacted] is provided for immunizing a host against the effects of the product of a target proto-oncogene, where the overexpression of the target proto-oncogene is associated with a malignancy. The cellular immunogen comprises host cells which have been transfected with at least one transgene construct comprising a transgene

cognate to the target proto-oncogene and a strong promoter to drive the expression of the transgene in the ***transfected*** ***cells*** . The transgene encodes a gene product which induces host immunoreactivity to host self-determinants of the product of the target proto-oncogene gene. The transgene may comprise, for example, wild-type or mutant retroviral oncogene DNA cognate to the target proto-oncogene; or wild-type or mutant proto-oncogene DNA of a species different from the host species. The cellular immunogen may be prepared from biopsied host cells, e.g. skin fibroblasts, which are stably or transiently transfected with the transgene construct containing the cognate transgene. The host cells transfected with the cognate transgene construct, are then returned to the body of the host to obtain expression of the cognate transgene in the host.

L9 ANSWER 4 OF 34 USPATFULL
AN 2001:233136 USPATFULL
TI Novel amphipathic aldehydes and their uses as adjuvants and immunoeffectors
IN Johnson, David A., Hamilton, MT, United States
PI US 2001053363 A1 20011220
AI US 2001-810915 A1 20010316 (9)
PRAI US 2000-190466P 20000317 (60)
DT Utility
FS APPLICATION
LREP TOWNSEND AND TOWNSEND AND CREW, TWO EMBARCADERO CENTER, EIGHTH FLOOR, SAN FRANCISCO, CA, 94111-3834
CLMN Number of Claims: 47
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 2531
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to novel aldehyde containing compounds and their uses as adjuvants and immunoeffectors.

L9 ANSWER 5 OF 34 USPATFULL
AN 2001:218004 USPATFULL
TI Cell surface molecule-induced macrophage activation
IN Tao, Weng, Lincoln, RI, United States
Wong, Shou, Cumberland, RI, United States
Hickey, William F., Lyme, NH, United States
Hammang, Joseph P., Barrington, RI, United States
Baetge, E. Edward, St. Sulpice, Switzerland
PI US 2001046490 A1 20011129
AI US 2001-761413 A1 20010116 (9)
RLI Continuation of Ser. No. US 2000-562544, filed on 2 May 2000, GRANTED, Pat. No. US 6225448 Division of Ser. No. US 1998-178869, filed on 26 Oct 1998, GRANTED, Pat. No. US 6197294
DT Utility
FS APPLICATION
LREP IVOR R. ELRIFI, Esq., Attorneys for Applicants, c/o MINTZ LEVIN, One Financial Center, Boston, MA, 02111
CLMN Number of Claims: 34
ECL Exemplary Claim: 1
DRWN 5 Drawing Page(s)
LN.CNT 1527
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides cells containing recombinant polynucleotides

coding for cell surface molecules that, when expressed in the cell, result in rejection of the cell by the host immune system. The invention also provides methods of using such cells, and capsules for delivery of biologically active molecules to a patient.

L9 ANSWER 6 OF 34 USPATFULL
AN 2001:194124 USPATFULL
TI Combinatorial enzymatic complexes
IN Nolan, Garry P., Menlo Park, CA, United States
Payan, Donald, Hillsborough, CA, United States
PA Rigel Pharmaceuticals, Inc. (U.S. corporation)
PI US 2001036638 A1 20011101
AI US 2001-789652 A1 20010220 (9)
RLI Division of Ser. No. US 1997-873601, filed on 12 Jun 1997, PENDING
DT Utility
FS APPLICATION
LREP FLEHR HOHBACH TEST, ALBRITTON & HERBERT LLP, Suite 3400, Four Embarcadero Center, San Francisco, CA, 94111-4187
CLMN Number of Claims: 26
ECL Exemplary Claim: 1
DRWN 5 Drawing Page(s)
LN.CNT 2249
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to the formation of novel in vivo combinatorial enzyme complexes for use in screening candidate drug agents for bioactivity.

L9 ANSWER 7 OF 34 USPATFULL
AN 2001:139156 USPATFULL
TI T cell receptor ligands and methods of using same
IN Germain, Ronald N., Potomac, MD, United States
Racioppi, Luigi, Naples, Italy
Ronchese-Le Gros, Franca, Wellington, New Zealand
PI US 2001016198 A1 20010823
AI US 2001-776520 A1 20010202 (9)
RLI Continuation of Ser. No. US 1999-293738, filed on 16 Apr 1999, ABANDONED Continuation of Ser. No. US 1997-858248, filed on 19 May 1997, GRANTED, Pat. No. US 5948409 Division of Ser. No. US 1993-4936, filed on 15 Jan 1993, GRANTED, Pat. No. US 5837477
DT Utility
FS APPLICATION
LREP KNOBBE MARTENS OLSON & BEAR LLP, 620 NEWPORT CENTER DRIVE, SIXTEENTH FLOOR, NEWPORT BEACH, CA, 92660
CLMN Number of Claims: 17
ECL Exemplary Claim: 1
DRWN 12 Drawing Page(s)
LN.CNT 1255
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention concerns TCR ligands with immunomodulatory properties, as well as methods of identifying such ligands and of using such ligands to modulate T cell effector responses.

L9 ANSWER 8 OF 34 USPATFULL
AN 2001:91501 USPATFULL
TI Green fluorescent protein fusions with random peptides
IN Anderson, David, San Bruno, CA, United States
Bogenberger, Jakob Maria, Menlo Park, CA, United States

PA Rigel Pharmaceuticals, Inc. (U.S. corporation)
PI US 2001003650 A1 20010614
AI US 2000-749959 A1 20001227 (9)
RLI Continuation of Ser. No. US 1998-169015, filed on 8 Oct 1998, GRANTED, Pat. No. US 6180343
DT Utility
FS APPLICATION
LREP Robin M. Silva, FLEHR HOHBACH TEST ALBRITTON & HERBERT LLP, Suite 3400, Four Embarcadero Center, San Francisco, CA, 94111-4187
CLMN Number of Claims: 25
ECL Exemplary Claim: 1
DRWN 3 Drawing Page(s)
LN.CNT 2537
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to the use of fluorescent proteins, particularly green fluorescent protein (GFP), in fusion constructs with random and defined peptides and peptide libraries, to increase the cellular expression levels, decrease the cellular catabolism, increase the conformational stability relative to linear peptides, and to increase the steady state concentrations of the random peptides and random peptide library members expressed in cells for the purpose of detecting the presence of the peptides and screening random peptide libraries. N-terminal, C-terminal, dual N- and C-terminal and one or more internal fusions are all contemplated. Novel fusions utilizing self-binding peptides to create a conformationally stabilized fusion domain are also contemplated.

L9 ANSWER 9 OF 34 USPATFULL
AN 2001:157795 USPATFULL
TI Anti-IgE antibodies and method of improving polypeptides
IN Lowman, Henry B., 400 San Juan Ave., El Granada, CA, United States 94018
Presta, Leonard G., 1900 Gough St. #206, San Francisco, CA, United States 94109
Jardieu, Paula M., 33 Hayward Ave. #110, San Mateo, CA, United States 94401-4319
Lowe, John, 396 Michelle La., Daly City, CA, United States 94080
PI US 6290957 B1 20010918
AI US 1999-296005 19990421 (9)
RLI Continuation of Ser. No. US 1997-887352, filed on 2 Jul 1997, now patented, Pat. No. US 5994511
DT Utility
FS GRANTED
EXNAM Primary Examiner: Saunders, David
LREP Svoboda, Craig G.
CLMN Number of Claims: 19
ECL Exemplary Claim: 1
DRWN 21 Drawing Figure(s); 19 Drawing Page(s)
LN.CNT 4910
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a method for adjusting the affuinity of a polypeptide to a target molecule by a combination of steps, including: (1) the identification of aspartyl residues which are prone to isomerization; (2) the substitution of alternative residues and screening the resulting mutants for affinity against the target molecule. In a preferred embodiment, the method of subtituting residues is affinity maturation with phage display (AMPD). In a further preferred

embodiment the polypeptide is an antibody and the target molecule is an antigen. In a further preferred embodiment, the antibody is anti-IgE and the target molecule is IgE. In another embodiment, the invention relates to an anti-IgE antibody having improved affinity to IgE.

L9 ANSWER 10 OF 34 USPATFULL
AN 2001:125564 USPATFULL
TI Melanoma antigens and their use in diagnostic and therapeutic methods
IN Kawakami, Yutaka, Rockville, MD, United States
Rosenberg, Steven A., Potomac, MD, United States
PA The United States of America as represented by the Department of Health and Human Services, Rockville, MD, United States (U.S. government)
PI US 6270778 B1 20010807
AI US 1999-267439 19990312 (9)
RLI Division of Ser. No. US 1998-73138, filed on 5 May 1998 Continuation-in-part of Ser. No. US 1995-417174, filed on 5 Apr 1995, now patented, Pat. No. US 5844075 Continuation-in-part of Ser. No. US 1994-231565, filed on 22 Apr 1994, now patented, Pat. No. US 5874560
DT Utility
FS GRANTED
EXNAM Primary Examiner: Huff, Sheela
LREP Morgan & Finnegan, L.L.P., Feiler, William S., Auth, Dorothy R.
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN 13 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 3383
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a nucleic acid sequence encoding a melanoma antigen recognized by T lymphocytes, designated MART-1. This invention further relates to bioassays using the nucleic acid sequence, protein or antibodies of this invention to diagnose, assess or prognoses a mammal afflicted with melanoma or metastata melanoma. This invention also provides immunogenic peptides derived from the MART-1 melanoma antigen and a second melanoma antigen designated gp100. This invention further provides immunogenic peptides derived from the MART-1 melanoma antigen or gp100 antigen which have been modified to enhance their immunogenicity. The proteins and peptides provided can serve as an immunogen or ***vaccine*** to prevent or treat melanoma.

L9 ANSWER 11 OF 34 USPATFULL
AN 2001:82751 USPATFULL
TI Induction of immune response to antigens expressed by recombinant adeno-associated virus
IN Kurtzman, Gary J., Menlo Park, CA, United States
Engelman, Edgar G., Atherton, CA, United States
Podsakoff, Greg M., Fullerton, CA, United States
Brockstedt, Dirk G., Palo Alto, CA, United States
PA Avigen, Inc., CA, United States (U.S. corporation)
PI US 6242426 B1 20010605
AI US 1998-121162 19980723 (9)
PRAI US 1997-53733P 19970725 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Hauda, Karen M.; Assistant Examiner: Beckerleg, Anne Marie S
LREP Madson & Metcalf, Chahine, Kenneth G., Thomson, Christina
CLMN Number of Claims: 8

ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 2301
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates generally to immunization methods using recombinant viral vectors. In particular, the invention relates to methods and compositions for immunizing a subject with a nucleic acid molecule encoding an antigen of interest, wherein the nucleic acid molecule is delivered to the subject via a recombinant AAV virion.

L9 ANSWER 12 OF 34 USPATFULL
AN 2001:78703 USPATFULL
TI Presentation of hydrophobic antigens to T-cells by CD1 molecules
IN Porcelli, Steven A., Bronx, NY, United States
Brenner, Michael B., Newton, MA, United States
Beckman, Evan M., Sudbury, MA, United States
Furlong, Stephen T., Wilmington, DE, United States
PA Brigham and Women's Hospital, Boston, MA, United States (U.S. corporation)
PI US 6238676 B1 20010529
AI US 1995-501600 19950712 (8)
RLI Continuation-in-part of Ser. No. US 1994-322980, filed on 13 Oct 1994, now patented, Pat. No. US 5679347 Continuation of Ser. No. US 1994-322979, filed on 13 Oct 1994, now patented, Pat. No. US 5853737 Continuation-in-part of Ser. No. WO 1994-US6991, filed on 21 Jun 1994 Continuation-in-part of Ser. No. US 1993-80072, filed on 21 Jun 1993, now abandoned Continuation-in-part of Ser. No. US 1992-989790, filed on 10 Dec 1992, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Chan, Christina Y.; Assistant Examiner: DiBrino, Marianne
LREP Wolf, Greenfield & Sacks, PC
CLMN Number of Claims: 28
ECL Exemplary Claim: 1
DRWN 63 Drawing Figure(s); 36 Drawing Page(s)
LN.CNT 2851
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Provided are CD-1 presented antigens, compositions, cells, inhibitors and methods relating to the use of hydrophobic antigen presentation by CD1 molecules, including:

methods for detecting the presence of a CD1-presented hydrophobic antigen in a sample;

methods for isolating such CD1-presented antigens and the isolated antigens;

vaccines containing CD1-presented antigens and vaccination methods;

methods of blocking CD1 antigen presentation;

methods of identifying and/or isolating CD1 blocking agents and the isolated CD1 blocking agents;

methods of inducing CD1 expression; and

T-cells for use in the methods disclosed.

L9 ANSWER 13 OF 34 USPATFULL
AN 2001:78697 USPATFULL
TI Compositions and methods employing a ligand for CD21 or CD19 for modulating the immune response to an antigen
IN Fearon, Douglas T., Cambridge, United Kingdom
Dempsey, Paul W., Cambridge, United Kingdom
PA Cambridge University Technical Services Limited, Cambridge, United Kingdom (non-U.S. corporation)
PI US 6238670 B1 20010529
WO 9617625 19960613
AI US 1997-849488 19971021 (8)
WO 1995-GB2851 19951206
19971021 PCT 371 date
19971021 PCT 102(e) date
PRAI GB 1994-24631 19941206
DT Utility
FS Granted
EXNAM Primary Examiner: Saunders, David; Assistant Examiner: DeCloux, Amy
LREP Flehr Hohbach Test Albritton & Herbert LLP, Trecartin, Richard F.
CLMN Number of Claims: 38
ECL Exemplary Claim: 1
DRWN 10 Drawing Figure(s); 10 Drawing Page(s)
LN.CNT 1084
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Described herein are compositions which modulate the immune response. In one aspect, a composition is described which comprises an antigen covalently linked to a ligand for CD21(CR2) or CD19. This antigen is not associated with a complement C3 fragment through an ester bond derived from the internal thioester of the complement C3 fragment.

L9 ANSWER 14 OF 34 USPATFULL
AN 2001:63825 USPATFULL
TI 1gG /transferrin receptor fusion protein
IN Tao, Weng, Lincoln, RI, United States
Wong, Shou, Cumberland, RI, United States
Hickey, William F., Lyme, NH, United States
Hammang, Joseph P., Barrington, RI, United States
Baetge, E. Edward, St. Sulpice, Switzerland
PA Neurotech S.A., Evry, France (non-U.S. corporation)
PI US 6225448 B1 20010501
AI US 2000-562544 20000502 (9)
RLI Division of Ser. No. US 1998-178869, filed on 26 Oct 1998
DT Utility
FS Granted
EXNAM Primary Examiner: Clark, Deborah J. R.; Assistant Examiner: Wilson, Michael C
LREP Mints, Levin, Cohn, Ferris, Glovsky and Popeo, P.C.
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN 5 Drawing Figure(s); 5 Drawing Page(s)
LN.CNT 1389
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides cells containing recombinant polynucleotides coding for cell surface molecules that, when expressed in the cell, result in rejection of the cell by the host immune system. The invention

also provides methods of using such cells, and capsules for delivery of biologically active molecules to a patient.

L9 ANSWER 15 OF 34 USPATFULL
AN 2001:32792 USPATFULL
TI Cell surface molecule-induced macrophage activation
IN Tao, Weng, Lincoln, RI, United States
Wong, Shou, Cumberland, RI, United States
Hickey, William F., Lyme, NH, United States
Hammang, Joseph P., Barrington, RI, United States
Baetge, E. Edward, St. Sulpice, Switzerland
PA Neurotech S.A., Evry, France (non-U.S. corporation)
PI US 6197294 B1 20010306
AI US 1998-178869 19981026 (9)
DT Utility
FS Granted
EXNAM Primary Examiner: LeGuyader, John L.; Assistant Examiner: Wilson, Michael C.
LREP Mintz,Levin,Cohn,Ferris, Glovsky and Popeo, P.C., Elrifi, Ivor R., Prince, John
CLMN Number of Claims: 6
ECL Exemplary Claim: 1
DRWN 5 Drawing Figure(s); 5 Drawing Page(s)
LN.CNT 1400
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention provides cells containing recombinant polynucleotides coding for cell surface molecules that, when expressed in the cell, result in rejection of the cell by the host immune system. The invention also provides methods of using such cells, and capsules for delivery of biologically active molecules to a patient.

L9 ANSWER 16 OF 34 USPATFULL
AN 2001:14201 USPATFULL
TI Green fluorescent protein fusions with random peptides
IN Anderson, David, San Bruno, CA, United States
Bogenberger, Jakob Maria, Menlo Park, CA, United States
PA Rigel Pharmaceuticals, Inc., S. San Francisco, CA, United States (U.S. corporation)
PI US 6180343 B1 20010130
AI US 1998-169015 19981008 (9)
DT Utility
FS Granted
EXNAM Primary Examiner: Brusca, John S.
LREP Flehr Hohbach Test Albritton & Herbert LLP
CLMN Number of Claims: 21
ECL Exemplary Claim: 1
DRWN 7 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 2522
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention relates to the use of fluorescent proteins, particularly green fluorescent protein (GFP), in fusion constructs with random and defined peptides and peptide libraries, to increase the cellular expression levels, decrease the cellular catabolism, increase the conformational stability relative to linear peptides, and to increase the steady state concentrations of the random peptides and random peptide library members expressed in cells for the purpose of detecting the presence of the peptides and screening random peptide libraries.

N-terminal, C-terminal, dual N- and C-terminal and one or more internal fusions are all contemplated. Novel fusions utilizing self-binding peptides to create a conformationally stabilized fusion domain are also contemplated.

L9 ANSWER 17 OF 34 USPATFULL
AN 2001:4887 USPATFULL
TI Anti-IgE antibodies and method of improving polypeptides
IN Lowman, Henry B., El Granada, CA, United States
Presta, Leonard G., San Francisco, CA, United States
Jardieu, Paula M., San Mateo, CA, United States
Lowe, John, Daly City, CA, United States
PA Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)
PI US 6172213 B1 20010109
AI US 1998-109207 19980630 (9)
PRAI US 1997-51554P 19970702 (60)
DT Patent
FS Granted
EXNAM Primary Examiner: Chan, Christina Y.; Assistant Examiner: Ewoldt, Gerald R.
LREP Svoboda, Craig G.
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN 23 Drawing Figure(s); 19 Drawing Page(s)
LN.CNT 4829
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a method for adjusting the affinity of a polypeptide to a target molecule by a combination of steps, including: (1) the identification of aspartyl residues which are prone to isomerization; (2) the substitution of alternative residues and screening the resulting mutants for affinity against the target molecule. In a preferred embodiment, the method of substituting residues is affinity maturation with phage display (AMPD). In a further preferred embodiment the polypeptide is an antibody and the target molecule is an antigen. In a further preferred embodiment, the antibody is anti-IgE and the target molecule is IgE. In another embodiment, the invention relates to an anti-IgE antibody having improved affinity to IgE.

L9 ANSWER 18 OF 34 USPATFULL
AN 2000:160777 USPATFULL
TI Methods for screening for transdominant intracellular effector peptides and RNA molecules
IN Nolan, Garry P., Palo Alto, CA, United States
Rothenberg, S. Michael, Palo Alto, CA, United States
PA Rigel Pharmaceuticals, Inc., Sunnyvale, CA, United States (U.S. corporation)
The Board of Trustees for the Leland Stanford Junior University, Palo Alto, CA, United States (U.S. corporation)
PI US 6153380 20001128
AI US 1997-789333 19970123 (8)
RLI Continuation of Ser. No. US 1996-589108, filed on 23 Jan 1996, now abandoned And a continuation of Ser. No. US 1996-589911, filed on 23 Jan 1996, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Chan, Christina Y.; Assistant Examiner: VanderVegt, F.

Pierre
LREP Flehr Hohbach Test Albritton & Herbert LLP, Silva, Robin M.
CLMN Number of Claims: 27
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 4 Drawing Page(s)
LN.CNT 4104
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods and compositions for screening for intracellular transdominant effector peptides and RNA molecules selected inside living cells from randomized pools are provided.

L9 ANSWER 19 OF 34 USPATFULL
AN 2000:156961 USPATFULL
TI Antigen presenting cells of the adipocyte lineage
IN Mosca, Joseph D., Ellicott City, MD, United States
PA Osiris Therapeutics, Inc., Baltimore, MD, United States (U.S. corporation)
PI US 6149906 20001121
AI US 1998-157008 19980918 (9)
PRAI US 1997-59690P 19970920 (60)
DT Utility
FS Granted
EXNAM Primary Examiner: Chan, Christina Y.; Assistant Examiner: Ewoldt, Gerald R.
LREP Olstein, Elliot M., Lillie, Raymond J.
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN 8 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 915
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Disclosed is a mesenchymal stem cell and/or cell of the adipocyte lineage that (i) has been modified to have at least one exogenous antigen bound to at least one primary surface molecule of said cell such that said at least one antigen can initiate an immune response and (ii) also expresses at least one co-stimulatory molecule. The antigen is preferably a protein, polypeptide, lipid or glycolipid. The primary surface molecule is MHC I, MHC II or CD1. Also disclosed is a method for stimulating presentation of at least one exogenous antigen fragment on a mesenchymal stem cell primary surface molecule by contacting a mesenchymal stem cell that is capable of expressing at least one co-stimulatory molecule with (i) an exogenous antigen or (ii) genetic material that codes for the exogenous antigen which the mesenchymal stem cell processes into it least one antigen fragment. The method can further include contacting the mesenchymal stem cell with interferon-.gamma.. Also disclosed are a method for determining the state of activation of a T lymphocyte population and a method for the treatment or prevention of a disease in an animal.

L9 ANSWER 20 OF 34 USPATFULL
AN 1999:155894 USPATFULL
TI Anti-IgE antibodies and methods of improving polypeptides
IN Lowman, Henry B., El Granada, CA, United States
Presta, Leonard G., San Francisco, CA, United States
Jardieu, Paula M., San Mateo, CA, United States
Lowe, John, Daly City, CA, United States
PA Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)

PI US 5994511 19991130
AI US 1997-887352 19970702 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Saunders, David
LREP Svoboda, Craig G.
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 21 Drawing Figure(s); 19 Drawing Page(s)
LN.CNT 5816
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to a method for adjusting the affinity of a polypeptide to a target molecule by a combination of steps, including: (1) the identification of aspartyl residues which are prone to isomerization; (2) the substitution of alternative residues and screening the resulting mutants for affinity against the target molecule. In a preferred embodiment, the method of subtituting residues is affinity maturation with phage display (AMPD). In a further preferred embodiment the polypeptide is an antibody and the target molecule is an antigen. In a further preferred embodiment, the antibody is anti-IgE and the target molecule is IgE. In another embodiment, the invention relates to an anti-IgE antibody having improved affinity to IgE.

L9 ANSWER 21 OF 34 USPATFULL
AN 1999:121222 USPATFULL
TI Engineered antigen presenting cells and methods for their use
IN Robinson, William S., Burlingame, CA, United States
PA Leland Stanford Junior University, Palo Alto, CA, United States (U.S. corporation)
PI US 5962320 19991005
AI US 1997-888360 19970703 (8)
RLI Continuation-in-part of Ser. No. US 663157
DT Utility
FS Granted
EXNAM Primary Examiner: Railey, II, Johnny F.
LREP Pennie & Edmonds LLP
CLMN Number of Claims: 26
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1364
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Autologous, heterologous or xenogeneic primary cells or cell lines are genetically modified ex vivo to render the cells capable of processing and presenting selected antigens to cells of the immune system of a subject, and to express different HLA molecules for matching to the HLA specificity of the subject. The cells are also modified to express immunoregulatory molecules for directing the immune response of the subject. The cells and cell lines are used in methods to treat infectious diseases or cancer, or to prevent infectious disease by inoculation into a host to activate T cells and induce an antigen-specific immune response, and in assays of the cytolytic activity of a subject's T cells. The cells can also be used to suppress an unwanted immune response of a subject to a selected antigen where the cells lack expression of a costimulation molecule needed for T cell activation.

L9 ANSWER 22 OF 34 USPATFULL

AN 1999:117284 USPATFULL
TI T-cell receptor ligands and methods of using same
IN Germain, Ronald N., Potomac, MD, United States
Racioppi, Luigi, Bethesda, MD, United States
PA The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
PI US 5958712 19990928
AI US 1997-858825 19970519 (8)
RLI Division of Ser. No. US 1993-4936, filed on 15 Jan 1993, now patented, Pat. No. US 5837477
DT Utility
FS Granted
EXNAM Primary Examiner: Saunders, David
LREP Knobbe Martens Olson & Bear, LLP
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN 18 Drawing Figure(s); 12 Drawing Page(s)
LN.CNT 1358
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention concerns TCR ligands with immunomodulatory properties, as well as methods of identifying such ligands and of using such ligands to modulate T cell effector responses.

L9 ANSWER 23 OF 34 USPATFULL
AN 1999:106089 USPATFULL
TI T cell receptor ligands and methods of using same
IN Germain, Ronald N., Potomac, MD, United States
Racioppi, Luigi, Bethesda, MD, United States
PA The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
PI US 5948409 19990907
AI US 1997-858248 19970519 (8)
RLI Division of Ser. No. US 1993-4936, filed on 15 Jan 1993, now patented, Pat. No. US 5837477
DT Utility
FS Granted
EXNAM Primary Examiner: Saunders, David
LREP Knobbe Martens Olson & Bear, LLP
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 18 Drawing Figure(s); 12 Drawing Page(s)
LN.CNT 1337
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention concerns TCR ligands with immunomodulatory properties, as well as methods of identifying such ligands and of using such ligands to modulate T cell effector responses.

L9 ANSWER 24 OF 34 USPATFULL
AN 1999:96476 USPATFULL
TI Methods of treating inflammation and compositions therefor
IN McFadden, D. Grant, Edmonton, Canada
Lucas, Alexandra, Edmonton, Canada
PA Viron Therapeutics, Inc., London, Canada (non-U.S. corporation)
PI US 5939525 19990817
AI US 1995-411043 19950327 (8)
DT Utility
FS Granted

EXNAM Primary Examiner: Tsang, Cecilia J.; Assistant Examiner: Delaney, Patrick R.
LREP Scully, Scott, Murphy & Presser
CLMN Number of Claims: 4
ECL Exemplary Claim: 1
DRWN 23 Drawing Figure(s); 15 Drawing Page(s)
LN.CNT 2356
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Compositions and methods for treating inflammatory cell infiltration in a tissue of a mammalian subject are provided. The method involves administering a therapeutically effective amount of SERP-1, SERP-1 analog or biologically active fragment thereof admixed with a pharmaceutically acceptable carrier to a subject in need of such treatment. Biologically active SERP-1 analogs are also provided. The compositions and methods of the present invention are useful for treating numerous inflammatory based diseases and injuries.

L9 ANSWER 25 OF 34 USPATFULL
AN 1999:92298 USPATFULL
TI AIDS therapy and ***vaccine***
IN Habeshaw, John Anthony, Harpenden, United Kingdom
Dalgleish, Angus George, London, United Kingdom
Hounsell, Elizabeth, Isleworth, United Kingdom
Bountiff, Lynne, Aylebury, United Kingdom
PA Retroscreen Limited, Whitechapel, United Kingdom (non-U.S. corporation)
PI US 5935579 19990810
AI US 1994-323686 19941014 (8)
RLI Continuation of Ser. No. US 1991-766366, filed on 25 Sep 1991, now abandoned
PRAI GB 1990-20999 19900925
GB 1990-22330 19901015
GB 1991-6540 19910327
DT Utility
FS Granted
EXNAM Primary Examiner: Eisenschenk, Frank C.; Assistant Examiner: Nelson, Brett
LREP Hale and Dorr LLP
CLMN Number of Claims: 9
ECL Exemplary Claim: 1
DRWN 10 Drawing Figure(s); 7 Drawing Page(s)
LN.CNT 3135
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides therapy and prophylaxis against HIV-induced AIDS, as well as methods for ascertaining the susceptibility of an individual to HIV-induced AIDS, the invention being based on the discovery that AIDS results from gp120 of HIV mimicking the antigen-presenting component of the immune system, thereby spuriously activating certain CD4+ T cells in susceptible individuals, leading to a condition similar to graft versus host disease, the condition being treatable by eliminating the responsible T cells, for example.

L9 ANSWER 26 OF 34 USPATFULL
AN 1999:72706 USPATFULL
TI Methods of treating inflammation and compositions therefor
IN McFadden, D. Grant, Edmonton, Canada
Lucas, Alexandra, Edmonton, Canada
PA Viron Therapeutics, Inc., London, Canada (non-U.S. corporation)

PI US 5917014 19990629
AI US 1995-468865 19950606 (8)
RLI Continuation of Ser. No. US 1995-411043, filed on 27 Mar 1995
DT Utility
FS Granted
EXNAM Primary Examiner: Tsang, Cecilia J.; Assistant Examiner: Delaney, Patrick R.
LREP Scully, Scott, Murphy & Presser
CLMN Number of Claims: 4
ECL Exemplary Claim: 1
DRWN 23 Drawing Figure(s); 15 Drawing Page(s)
LN.CNT 2074
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Compositions and methods for treating inflammatory cell infiltration in a tissue of a mammalian subject are provided. The method involves administering a therapeutically effective amount of SERP-1, SERP-1 analog or biologically active fragment thereof admixed with a pharmaceutically acceptable carrier to a subject in need of such treatment. Biologically active SERP-1 analogs are also provided. The compositions and methods of the present invention are useful for treating numerous inflammatory based diseases and injuries.

L9 ANSWER 27 OF 34 USPATFULL
AN 1999:24776 USPATFULL
TI Melanoma antigens and their use in diagnostic and therapeutic methods
IN Kawakami, Yutaka, Rockville, MD, United States
Rosenberg, Steven A., Potomac, MD, United States
PA The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
PI US 5874560 19990223
AI US 1994-231565 19940422 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Huff, Sheela
LREP Morgan & Finnegan, L.L.P.
CLMN Number of Claims: 1
ECL Exemplary Claim: 1
DRWN 13 Drawing Figure(s); 9 Drawing Page(s)
LN.CNT 2830
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a nucleic acid sequence encoding a melanoma antigen recognized by T lymphocytes, designated MART-1. This invention further relates to bioassays using the nucleic acid sequence, protein or antibodies of this invention to diagnose, assess or prognoses a mammal afflicted with melanoma or metastata melanoma. This invention also provides immunogenic peptides derived from the MART-1 melanoma antigen and a second melanoma antigen designated gp100. The proteins and peptides provided can serve as an immunogen or ***vaccine*** to prevent or treat melanoma.

L9 ANSWER 28 OF 34 USPATFULL
AN 1999:18729 USPATFULL
TI Recombinant vaccines to break self-tolerance
IN Rock, Edwin P., 4535 Hawthorne St., Washington, DC, United States 20016
PI US 5869057 19990209
AI US 1997-944982 19971007 (8)
RLI Continuation of Ser. No. US 1995-472455, filed on 7 Jun 1995, now

abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Achutamurthy, Ponnathapura; Assistant Examiner: Bui, Phuong T.
LREP Keil & Weinkauf
CLMN Number of Claims: 5
ECL Exemplary Claim: 1
DRWN 20 Drawing Figure(s); 12 Drawing Page(s)
LN.CNT 2000
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB This invention relates to vaccines, specifically to the use of recombinant DNA technology to immunize against self proteins and to induce antibody against self protein in mammals. A process is described in which DNA sequences encoding a microbial gene product and a self gene protein are joined and expressed by means of a suitable DNA vector and a non-pathogenic microbial strain. The present invention further relates to the isolation and purification of a fusion peptide combining the non-toxic B subunit of an enterotoxigenic strain of E. coli (LTB) with the carboxyl terminal peptide (CTP) of human chorionic gonadotropin (hCG), as well as to the use of this fusion protein for immunological prophylaxis and therapy.

L9 ANSWER 29 OF 34 USPATFULL
AN 1998:162012 USPATFULL
TI Method for inducing a CD1-restricted immune response
IN Modlin, Robert L., Sherman Oaks, CA, United States
Sieling, Peter A., Malibu, CA, United States
Brenner, Michael B., Brookline, MA, United States
Porcelli, Steven A., Brighton, MA, United States
Brennan, Patrick J., Fort Collins, CO, United States
PA Brigham and Women's Hospital, Boston, MA, United States (U.S. corporation)
University of California, Los Angeles, Los Angeles, CA, United States (U.S. corporation)
Colorado State University Research Foundation, Fort Collins, CO, United States (U.S. corporation)
PI US 5853737 19981229
AI US 1994-322979 19941013 (8)
RLI Continuation-in-part of Ser. No. US 1993-80072, filed on 19 Jun 1993, now abandoned which is a continuation-in-part of Ser. No. US 1992-989790, filed on 19 Dec 1992, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Cunningham, Thomas M.
LREP Hamilton, Brook, Smith & Reynolds, P.C.
CLMN Number of Claims: 60
ECL Exemplary Claim: 1
DRWN 55 Drawing Figure(s); 38 Drawing Page(s)
LN.CNT 2536
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is based on the observation that CD1 functions to present foreign and autoimmune antigens to a select subpopulation of T-cells. Based on this observation, the present invention provides methods for detecting the presence of a CD1-presented antigen in a sample, methods for purifying CD1-presented antigens, vaccines containing CD1-presented antigens, methods of blocking CD1 antigen

presentation, methods of identifying and/or isolating CD1 blocking agents, methods of inducing CD1 expression, and T-cell lines for use in the methods disclosed herein. The CD1-presented antigens of the invention, unlike MHC-presented antigens, are non-polypeptide hydrophobic antigens. In particular, a CD1-presented antigen isolated from several mycobacterial species is a lipoarabinomannan (LAM).

L9 ANSWER 30 OF 34 USPATFULL
AN 1998:151074 USPATFULL
TI Melanoma antigens and their use in diagnostic and therapeutic methods
IN Kawakami, Yutaka, Rockville, MD, United States
Rosenberg, Steven A., Potomac, MD, United States
PA The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
PI US 5844075 19981201
AI US 1995-417174 19950405 (8)
RLI Continuation-in-part of Ser. No. US 1994-231565, filed on 22 Apr 1994
DT Utility
FS Granted
EXNAM Primary Examiner: Huff, Sheela
LREP Morgan & Finnegan, L.L.P.
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN 18 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 4154
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides a nucleic acid sequence encoding a melanoma antigen recognized by T lymphocytes, designated MART-1. This invention further relates to bioassays using the nucleic acid sequence, protein or antibodies of this invention to diagnose, assess or prognoses a mammal afflicted with melanoma or metastata melanoma. This invention also provides immunogenic peptides derived from the MART-1 melanoma antigen and a second melanoma antigen designated gp100. This invention further provides immunogenic peptides derived from the MART-1 melanoma antigen or gp100 antigen which have been modified to enhance their immunogenicity. The proteins and peptides provided can serve as an immunogen or ***vaccine*** to prevent or treat melanoma.

L9 ANSWER 31 OF 34 USPATFULL
AN 1998:143882 USPATFULL
TI T cell receptor ligands and methods of using same
IN Germain, Ronald N., Potomac, MD, United States
Racioppi, Luigi, Bethesda, MD, United States
Gros, Franca Ronchese-Le, Brooklyn, New Zealand
PA The United States of America as represented by the Department of Health and Human Services, Washington, DC, United States (U.S. government)
PI US 5837477 19981117
AI US 1993-4936 19930115 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Saunders, David
LREP Knobbe, Martens, Olson & Bear, LLP
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 18 Drawing Figure(s); 11 Drawing Page(s)
LN.CNT 1311
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention concerns TCR ligands with immunomodulatory properties, as well as methods of identifying such ligands and of using such ligands to modulate T cell effector responses.

L9 ANSWER 32 OF 34 USPATFULL
AN 1998:64956 USPATFULL
TI Immunogenic cancer proteins and peptides and methods of use
IN Calenoff, Emanuel, Chicago, IL, United States
PA Northwestern University, Evanston, IL, United States (U.S. corporation)
PI US 5763164 19980609
AI US 1994-191338 19940203 (8)
RLI Continuation-in-part of Ser. No. US 1993-49698, filed on 16 Apr 1993, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Jones, W. Gary; Assistant Examiner: Rees, Dianne
LREP Brinks Hofer Gilson & Lione
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 13 Drawing Figure(s); 13 Drawing Page(s)
LN.CNT 2928
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to tumor specific antigens and functional proteins of a tumor cell preparable by identifying protein presents in the tumor cell that are selectively immunogenic for tumor patients. The present invention still further provides a process of making a peptide library of tumor specific humoral antigens, a process of increasing the immunogenic specificity of a tumor-associated antigen, an assay kit for detecting the presence of an antibody immunoreactive with a tumor-specific antigen, and a process of making T cells sensitized to a tumor-specific antigen.

L9 ANSWER 33 OF 34 USPATFULL
AN 97:96843 USPATFULL
TI Methods and devices for immunizing a host against tumor-associated antigens through administration of naked polynucleotides which encode tumor-associated antigenic peptides
IN Carson, Dennis A., Del Mar, CA, United States
Raz, Eyal, San Diego, CA, United States
PA The Regents of the University of California, Alameda, CA, United States (U.S. corporation)
PI US 5679647 19971021
AI US 1994-334260 19941103 (8)
DCD 20141101
RLI Continuation-in-part of Ser. No. US 1993-112440, filed on 26 Aug 1993, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Eisenschenk, Frank C.
LREP Fish & Richardson P.C.
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 31 Drawing Figure(s); 18 Drawing Page(s)
LN.CNT 2375
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The invention is directed to methods for introducing biologically active peptides into a host by administration of polynucleotides which

operatively encode for the peptide of interest. In a preferred embodiment of the invention, a host who has been identified as having a tumor bearing at least one tumor-associated antigen is the recipient of a polynucleotide which operatively encodes for a foreign mimic of the tumor-associated antigen or a mutation of the ***self*** - ***antigen*** . The antigen-encoding polynucleotides are administered to host tissues which have a high concentration of antigen presenting cells in them relative to other host tissues. The method is particularly useful in treating cancer through induction of antigen-specific cytotoxic T lymphocytes in the host for lysis of tumor cells bearing the antigen. Devices and compositions for use in the methods of the invention are also described.

L9 ANSWER 34 OF 34 USPATFULL
AN 97:96556 USPATFULL
TI Methods of isolating CD1-presented antigens, vaccines comprising CD1-presented antigens, and cell lines for use in said methods
IN Porcelli, Steven A., Brighton, MA, United States
Brenner, Michael B., Brookline, MA, United States
Beckman, Evan M., Brookline, MA, United States
PA Brigham and Women's Hospital, Boston, MA, United States (U.S. corporation)
PI US 5679347 19971021
AI US 1994-322980 19941013 (8)
RLI Continuation-in-part of Ser. No. US 1993-80072, filed on 21 Jun 1993, now abandoned which is a continuation-in-part of Ser. No. US 1992-989790, filed on 10 Dec 1992, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Cunningham, Thomas M.
LREP Hamilton, Brook, Smith & Reynolds, P.C.
CLMN Number of Claims: 20
ECL Exemplary Claim: 1
DRWN 55 Drawing Figure(s); 38 Drawing Page(s)
LN.CNT 2422
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention is based on the observation that CD1 functions to present foreign and autoimmune antigens to a select subpopulation of T-cells. Based on this observation, the present invention provides methods for detecting the presence of a CD1-presented antigen in a sample, methods for purifying CD1-presented antigens, vaccines containing CD1-presented antigens, methods of blocking CD1 antigen presentation, methods of identifying and/or isolating CD1 blocking agents, methods of inducing CD1 expression, and T-cell lines for use in the methods disclosed herein. The CD1-presented antigens of the invention, unlike MHC-presented antigens, are non-polypeptide hydrophobic antigens. In particular, a CD1-presented antigen isolated from several mycobacterial species is a mycolic acid (MA).

=> s epitope over-expressed by tumo!r
L10 0 EPITOPE OVER-EXPRESSED BY TUMO!R

=> s epitope over-expressed by tumo!r cells
L11 0 EPITOPE OVER-EXPRESSED BY TUMO!R CELLS

=> s (tumo!r cell epitope) and (transfected)

```
L12          0 (TUMO!R CELL EPITOPE) AND (TRANSFECTED)

=> s HER2 and (transfected cell)
L13         96 HER2 AND (TRANSFECTED CELL)

=> dup rem l13
PROCESSING COMPLETED FOR L13
L14          79 DUP REM L13 (17 DUPLICATES REMOVED)

=> d his

     (FILE 'HOME' ENTERED AT 16:19:18 ON 03 APR 2002)

     FILE 'USPATFULL' ENTERED AT 16:24:00 ON 03 APR 2002
L1           10 S (EXPRESSION IN PLANT!) AND (ANTI-IDIOTYP?)
L2           10 DUP REM L1 (0 DUPLICATES REMOVED)

     FILE 'MEDLINE, BIOSIS, EMBASE, SCISEARCH, WPIDS, USPATFULL, CANCERLIT'
     ENTERED AT 16:26:36 ON 03 APR 2002
L3          210 S VACCINE AND (SELF-ANTIGEN)
L4           67 S L3(10A)(TRANSFORMED OR TRANSFECTED)
L5           18 S L3 AND (TRANSFORMED CELLS)
L6            0 S L3 AND (TRANFECTED CELL!)
L7           19 S L3 AND (TRANSFECTED CELL!)
L8           34 S L5 OR L7
L9           34 DUP REM L8 (0 DUPLICATES REMOVED)
L10           0 S EPITOPE OVER-EXPRESSED BY TUMO!R
L11           0 S EPITOPE OVER-EXPRESSED BY TUMO!R CELLS
L12           0 S (TUMO!R CELL EPITOPE) AND (TRANSFECTED)
L13          96 S HER2 AND (TRANSFECTED CELL)
L14          79 DUP REM L13 (17 DUPLICATES REMOVED)

=> s l14 and (plant expression )
L15          0 L14 AND (PLANT EXPRESSION )

=> s l14 and (transformed plant)
L16          0 L14 AND (TRANSFORMED PLANT)

=> s l14 and (recombinant plant)
L17          0 L14 AND (RECOMBINANT PLANT)

=> s l14 5a (recombinant plant)
MISSING OPERATOR L14 5A
The search profile that was entered contains terms or
nested terms that are not separated by a logical operator.

=> s l14(5w)(recombinant plant)
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L130(5W)(RECOMBINA'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L132(5W)(RECOMBINA'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L134(5W)(RECOMBINA'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L136(5W)(RECOMBINA'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L138(5W)(RECOMBINA'
```

PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L140(5W)(RECOMBINA'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L142(5W)(RECOMBINA'
L18 0 L14(5W)(RECOMBINANT PLANT)

=> s vaccine(5w)(recombinant plant)
L19 4 VACCINE(5W)(RECOMBINANT PLANT)

=> dup rem l19
PROCESSING COMPLETED FOR L19
L20 1 DUP REM L19 (3 DUPLICATES REMOVED)

=> d l20 bib ab

L20 ANSWER 1 OF 1 MEDLINE DUPLICATE 1
AN 2001328100 MEDLINE
DN 21289395 PubMed ID: 11395200
TI Inactivated recombinant plant virus protects dogs from a lethal challenge with canine parvovirus.
AU Langeveld J P; Brennan F R; Martinez-Torrecuadrada J L; Jones T D; Boshuizen R S; Vela C; Casal J I; Kamstrup S; Dalsgaard K; Meloen R H; Bendig M M; Hamilton W D
CS Institute for Animal Science and Health (ID-Lelystad), PO Box 65 NL-8200 AB, Lelystad, The Netherlands.
SO VACCINE, (2001 Jun 14) 19 (27) 3661-70.
Journal code: X6O; 8406899. ISSN: 0264-410X.
CY England: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200109
ED Entered STN: 20010924
Last Updated on STN: 20010924
Entered Medline: 20010920
AB A ***vaccine*** based upon a ***recombinant*** ***plant*** virus (CPMV-PARVO1), displaying a peptide derived from the VP2 capsid protein of canine parvovirus (CPV), has previously been described. To date, studies with the vaccine have utilized viable plant chimaeric particles (CVPs). In this study, CPMV-PARVO1 was inactivated by UV treatment to remove the possibility of replication of the recombinant plant virus in a plant host after manufacture of the vaccine. We show that the inactivated CVP is able to protect dogs from a lethal challenge with CPV following parenteral immunization with the vaccine. Dogs immunized with the inactivated CPMV-PARVO1 in adjuvant displayed no clinical signs of disease and shedding of CPV in faeces was limited following CPV challenge. All immunized dogs elicited high titres of peptide-specific antibody, which neutralized CPV in vitro. Levels of protection, virus shedding and VP2-specific antibody were comparable to those seen in dogs immunized with the same VP2- peptide coupled to keyhole limpet hemocyanin (KLH). Since plant virus-derived vaccines have the potential for cost-effective manufacture and are not known to replicate in mammalian cells, they represent a viable alternative to current replicating vaccine vectors for development of both human and veterinary vaccines.

=> s (immunoglobulin)(10w)plant!

L21 42 (IMMUNOGLOBULIN)(10W) PLANT!

=> dup rem l21
PROCESSING COMPLETED FOR L21
L22 22 DUP REM L21 (20 DUPLICATES REMOVED)

=> d l22 1-22 bib ab

L22 ANSWER 1 OF 22 USPATFULL
AN 2001:178845 USPATFULL
TI Method for producing immunoglobulins containing protection proteins in plants and their use
IN Hiatt, Andrew C., San Diego, CA, United States
Ma, Julian K.-C., London, United Kingdom
Lehner, Thomas, Herts, United Kingdom
Mostov, Keith E., San Francisco, CA, United States
PA Planet Biotechnology, Inc., Kensington, CA, United States (U.S. corporation)
PI US 6303341 B1 20011016
AI US 1999-312157 19990514 (9)
RLI Continuation of Ser. No. US 1995-434000, filed on 4 May 1995, now patented, Pat. No. US 6046037 Continuation-in-part of Ser. No. US 1994-367395, filed on 30 Dec 1994, now abandoned
DT Utility
FS GRANTED
EXNAM Primary Examiner: Benzion, Gary
CLMN Number of Claims: 53
ECL Exemplary Claim: 1
DRWN 1 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 3418
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The immunoglobulins of the present invention are useful therapeutic immunoglobulins against mucosal pathogens such as S. mutans. The immunoglobulins contain a protection protein that protects the immunoglobulins in the mucosal environment.

The invention also includes the greatly improved method of producing immunoglobulins in plants by producing the protection protein in the same cell as the other components of the immunoglobulins. The components of the immunoglobulin are assembled at a much improved efficiency. The method of the invention allows the assembly and high efficiency production of such complex molecules.

The invention also contemplates the production of immunoglobulins containing protection proteins in a variety of cells, including plant cells, that can be selected for useful additional properties. The use of immunoglobulins containing protection proteins as therapeutic antibodies against mucosal and other pathogens is also contemplated.

L22 ANSWER 2 OF 22 USPATFULL
AN 2001:125782 USPATFULL
TI Control of fruit ripening and senescence in plants
IN Keinan, Ehud, Timrat, Israel
Itzhaky, Harel, Atlit, Israel
Aboud-Pirak, Esther, Kiryat Tivon, Israel
Gepstein, Shimon, Haifa, Israel
PA Vitality Biotechnologies, Inc., Orangeburg, NY, United States (U.S.

corporation)
PI US 6271009 B1 20010807
AI US 1999-245736 19990208 (9)
DT Utility
FS GRANTED
EXNAM Primary Examiner: Patterson, Jr., Charles L.
LREP Friedman, Mark M.
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN 4 Drawing Figure(s); 2 Drawing Page(s)
LN.CNT 1109
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Hapten and antigen designed for eliciting catalytic antibodies effective in inhibiting the ethylene production pathway in plants by deactivating a precursor thereof either by decomposition or derivatization. Catalytic antibodies effective in inhibiting the ethylene production pathway in plants by deactivating a precursor thereof. Genes encoding for such catalytic antibodies and plants and cells expressing these genes and producing the catalytic antibodies for controlling the ripening of fruits and vegetables, as well as for controlling senescence of plant tissue.

L22 ANSWER 3 OF 22 MEDLINE DUPLICATE 1
AN 2001199943 MEDLINE
DN 21183142 PubMed ID: 11289507
TI Assembly and plasma membrane targeting of recombinant ***immunoglobulin*** chains in ***plants*** with a murine immunoglobulin transmembrane sequence.
AU Vine N D; Drake P; Hiatt A; Ma J K
CS Department of Oral Medicine, Guy's Hospital, London, UK.
SO PLANT MOLECULAR BIOLOGY, (2001 Jan) 45 (2) 159-67.
Journal code: A6O; 9106343. ISSN: 0167-4412.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200104
ED Entered STN: 20010502
Last Updated on STN: 20010502
Entered Medline: 20010426
AB The cDNA encoding a full-length murine immunoglobulin gamma 1 heavy chain with its native leader sequence, transmembrane and intracellular domains was introduced into transgenic plants. Transformed plants expressed the recombinant polypeptide, but, in contrast to plants expressing the heavy chain without transmembrane sequence, the protein appeared to be associated with a plant cell membrane. Extraction of the membrane-associated heavy chain required the presence of a non-ionic detergent, and immunofluorescence studies of protoplasts demonstrated surface expression of membrane Ig heavy chain on up to 40% of the cells from a transgenic leaf. In plants expressing both the membrane Ig heavy chain and its partner light chain, functional antibody was also localised to the plant cell membrane and retention of the heavy chain at this site appeared to have no effect on the efficiency of antibody assembly. This approach of localising and accumulating recombinant antibody in cell membranes may have a number of applications, including passive immunisation against plant pathogens.

L22 ANSWER 4 OF 22 USPATFULL
AN 2000:80568 USPATFULL
TI Method for producing antibodies in plant cells
IN Russell, David R., Madison, WI, United States
Fuller, James T., Oregon, WI, United States
PA Monsanto Company, St. Louis, MO, United States (U.S. corporation)
PI US 6080560 20000627
AI US 1994-279772 19940725 (8)
DT Utility
FS Granted
EXNAM Primary Examiner: Smith, Lynette R. F.; Assistant Examiner: Haas, Thomas
LREP McKenna & Cuneo LLP
CLMN Number of Claims: 14
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 793
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A method for producing antibodies in plant cells including the steps of providing a genetic construct that encodes a secretable mammalian single chain antibody, delivering copies of the construct into a liquid suspension culture of tobacco cells, selecting for cells that have acquired the genetic construct, allowing the antibody to accumulate in the liquid to a concentration over 25 mg/l and isolating the antibody away from the tobacco cells.

L22 ANSWER 5 OF 22 USPATFULL
AN 2000:40882 USPATFULL
TI Method for producing immunoglobulins containing protection proteins in plants and their use
IN Hiatt, Andrew C., 660 Torrance St., San Diego, CA, United States 92103
Ma, Julian K.-C., 81 Grierson Road, London, United Kingdom SE231PE
Lehner, Thomas, 2 Wood Ride Hadley Wood, Barnet, Herts, United Kingdom EN40LL
Mostov, Keith E., 1975 Funston Ave., San Francisco, CA, United States 94116
PI US 6046037 20000404
AI US 1995-434000 19950504 (8)
RLI Continuation-in-part of Ser. No. US 1994-367395, filed on 30 Dec 1994, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Smith, Lynette F.; Assistant Examiner: Haas, Thomas
LREP Lyon & Lyon LLP
CLMN Number of Claims: 24
ECL Exemplary Claim: 1
DRWN 1 Drawing Figure(s); 1 Drawing Page(s)
LN.CNT 4923
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The immunoglobulins of the present invention are useful therapeutic immunoglobulins against mucosal pathogens such as S. mutans. The immunoglobulins contain a protection protein that protects the immunoglobulins in the mucosal environment.

The invention also includes the greatly improved method of producing immunoglobulins in plants by producing the protection protein in the same cell as the other components of the immunoglobulins. The components of the immunoglobulin are assembled at a much improved efficiency. The

method of the invention allows the assembly and high efficiency production of such complex molecules.

The invention also contemplates the production of immunoglobulins containing protection proteins in a variety of cells, including plant cells, that can be selected for useful additional properties. The use of immunoglobulins containing protection proteins as therapeutic antibodies against mucosal and other pathogens is also contemplated.

L22 ANSWER 6 OF 22 MEDLINE DUPLICATE 2
AN 2000498110 MEDLINE
DN 20398342 PubMed ID: 10938364
TI Assembly, secretion, and vacuolar delivery of a hybrid ***immunoglobulin*** in ***plants*** .
AU Frigerio L; Vine N D; Pedrazzini E; Hein M B; Wang F; Ma J K; Vitale A
CS Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom.
SO PLANT PHYSIOLOGY, (2000 Aug) 123 (4) 1483-94.
Journal code: P98; 0401224. ISSN: 0032-0889.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200010
ED Entered STN: 20001027
Last Updated on STN: 20001027
Entered Medline: 20001018
AB Secretory immunoglobulin (Ig) A is a decameric Ig composed of four alpha-heavy chains, four light chains, a joining (J) chain, and a secretory component (SC). The heavy and light chains form two tetrameric Ig molecules that are joined by the J chain and associate with the SC. Expression of a secretory monoclonal antibody in tobacco (Nicotiana tabacum) has been described: this molecule (secretory IgA/G [SIgA/G]) was modified by having a hybrid heavy chain sequence consisting of IgG gamma-chain domains linked to constant region domains of an IgA alpha-chain. In tobacco, about 70% of the protein assembles to its final, decameric structure. We show here that SIgA/G assembly and secretion are slow, with only approximately 10% of the newly synthesized molecules being secreted after 24 h and the bulk probably remaining in the endoplasmic reticulum. In addition, a proportion of SIgA/G is delivered to the vacuole as at least partially assembled molecules by a process that is blocked by the membrane traffic inhibitor brefeldin A. Neither the SC nor the J chain are responsible for vacuolar delivery, because IgA/G tetramers have the same fate. The parent IgG tetrameric molecule, containing wild-type gamma-heavy chains, is instead secreted rapidly and efficiently. This strongly suggests that intracellular retention and vacuolar delivery of IgA/G is due to the alpha-domains present in the hybrid alpha/gamma-heavy chains and indicates that the plant secretory system may partially deliver to the vacuole recombinant proteins expected to be secreted.

L22 ANSWER 7 OF 22 USPATFULL
AN 1999:117748 USPATFULL
TI Transgenic plants expressing assembled secretory antibodies
IN Hein, Mich B., Fallbrook, CA, United States
Hiatt, Andrew, San Diego, CA, United States
Ma, Julian K-C, London, United Kingdom
PA The Scripps Research Institute, La Jolla, CA, United States (U.S.

corporation)
PI US 5959177 19990928
AI US 1996-642406 19960503 (8)
RLI Continuation-in-part of Ser. No. US 1992-971951, filed on 5 Nov 1992, now patented, Pat. No. US 5639947 which is a continuation of Ser. No. US 1990-591823, filed on 2 Oct 1990, now patented, Pat. No. US 5202422 which is a continuation-in-part of Ser. No. US 1989-427765, filed on 27 Oct 1989, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Robinson, Douglas W.; Assistant Examiner: Haas, Thomas
LREP Fitting, Thomas, Holmes, Emily
CLMN Number of Claims: 12
ECL Exemplary Claim: 1
DRWN 10 Drawing Figure(s); 8 Drawing Page(s)
LN.CNT 4721
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention relates to expression and assembly of foreign multimeric proteins--e.g., antibodies--in plants, as well as to transgenic plants that express such proteins. In one of several preferred embodiments, the generation and assembly of functional secretory antibodies in plants is disclosed. The invention also discloses compositions produced by the transgenic plants of the present invention and methods of using same.

L22 ANSWER 8 OF 22 USPATFULL
AN 1999:27437 USPATFULL
TI Method for producing DNA encoding cystic fibrosis transmembrane conductance regulator (CFTR) protein in E. coli
IN Gregory, Richard J., Carlsbad, CA, United States
PA Genzyme Corporation, Framingham, MA, United States (U.S. corporation)
PI US 5876974 19990302
AI US 1994-298522 19940830 (8)
RLI Continuation of Ser. No. US 1993-87132, filed on 2 Jul 1993 which is a continuation of Ser. No. US 1990-613592, filed on 15 Nov 1990, now abandoned which is a continuation-in-part of Ser. No. US 1990-589295, filed on 27 Sep 1990, now abandoned which is a continuation-in-part of Ser. No. US 1990-488307, filed on 5 Mar 1990, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Carlson, Karen C.
LREP Baker & Botts, L.L.P
CLMN Number of Claims: 8
ECL Exemplary Claim: 1
DRWN 26 Drawing Figure(s); 23 Drawing Page(s)
LN.CNT 1815
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A pharmaceutical composition comprising a vector itself comprising a purified and isolated DNA sequence consisting essentially of a DNA sequence encoding a polypeptide having an amino acid sequence sufficiently duplicative of CFTR to allow possession of the biological property of correction of a defect in epithelial cell anion channel regulation.

L22 ANSWER 9 OF 22 MEDLINE DUPLICATE 3
AN 1999110954 MEDLINE
DN 99110954 PubMed ID: 9892697

TI Rapid production of specific vaccines for lymphoma by expression of the tumor-derived single-chain Fv epitopes in tobacco plants.
AU McCormick A A; Kumagai M H; Hanley K; Turpen T H; Hakim I; Grill L K; Tuse D; Levy S; Levy R
CS Biosource Technologies, Inc., 3333 Vacavalley Parkway, Suite 1000, Vacaville, CA 95688, USA.
NC AI37219 (NIAID)
CA33399 (NCI)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Jan 19) 96 (2) 703-8.
Journal code: PV3; 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199903
ED Entered STN: 19990326
Last Updated on STN: 19990326
Entered Medline: 19990316
AB Rapid production of protein-based tumor-specific vaccines for the treatment of malignancies is possible with the plant-based transient expression system described here. We created a modified tobamoviral vector that encodes the idiotype-specific single-chain Fv fragment (scFv) of the ***immunoglobulin*** from the 38C13 mouse B cell lymphoma. Infected Nicotiana benthamiana ***plants*** contain high levels of secreted scFv protein in the extracellular compartment. This material reacts with an anti-idiotype antibody by Western blotting, ELISA, and affinity chromatography, suggesting that the plant-produced 38C13 scFv protein is properly folded in solution. Mice vaccinated with the affinity-purified 38C13 scFv generate >10 micrograms/ml anti-idiotype immunoglobulins. These mice were protected from challenge by a lethal dose of the syngeneic 38C13 tumor, similar to mice immunized with the native 38C13 IgM-keyhole limpet hemocyanin conjugate vaccine. This rapid production system for generating tumor-specific protein vaccines may provide a viable strategy for the treatment of non-Hodgkin's lymphoma.

L22 ANSWER 10 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:155453 BIOSIS
DN PREV200000155453
TI Expression of a murine ***immunoglobulin*** with native transmembrane sequence in transgenic ***plants*** .
AU Vine, N. D. (1); Ma, J. K.-C. (1)
CS (1) Department of Oral Medicine and Pathology, GKT Institute for Medicine and Dentistry, London, SE1 9RT UK
SO Immunology., (Dec., 1999) Vol. 98, No. suppl. 1, pp. 148.
Meeting Info.: Joint Congress of the British Society for Immunology and the British Society for Allergy & Clinical Immunology. Harrogate, England, UK November 30-December 03, 1999 British Society for Allergy & Clinical Immunology
. ISSN: 0019-2805.
DT Conference
LA English
SL English

L22 ANSWER 11 OF 22 USPATFULL
AN 97:91555 USPATFULL
TI Methods and therapeutic compositions for treating cystic fibrosis

IN Cheng, Seng Hing, Wellesley, MA, United States
Fang, Shaona Lee, Sudbury, MA, United States
Hoppe, IV, Henry, Acton, MA, United States
Smith, Alan Edward, Dover, MA, United States
PA Genzyme Corporation, Cambridge, MA, United States (U.S. corporation)
PI US 5674898 19971007
AI US 1993-72708 19930607 (8)
RLI Continuation-in-part of Ser. No. US 1992-935603, filed on 26 Aug 1992, now abandoned which is a continuation-in-part of Ser. No. US 1990-613592, filed on 15 Nov 1990, now abandoned 76 Ser. No. US 1990-589295, filed on 27 Sep 1990, now abandoned which is a continuation-in-part of Ser. No. US 1990-488307, filed on 5 Mar 1990, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: O'Sullivan, Peter
CLMN Number of Claims: 34
ECL Exemplary Claim: 1
DRWN 22 Drawing Figure(s); 17 Drawing Page(s)
LN.CNT 2257
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB Methods and compositions for treating Cystic Fibrosis by mobilizing mutant forms of CFTR, which retain at least some functional activity, to the plasma membrane where they can mediate chloride ion transport are disclosed.

L22 ANSWER 12 OF 22 USPATFULL
AN 97:52192 USPATFULL
TI Compositions containing glycopolypeptide multimers and methods of making same in plants
IN Hiatt, Andrew C., San Diego, CA, United States
Hein, Mich B., Fallbrook, CA, United States
PA The Scripps Research Institute, La Jolla, CA, United States (U.S. corporation)
PI US 5639947 19970617
AI US 1992-971951 19921105 (7)
RLI Continuation of Ser. No. US 1990-591823, filed on 2 Oct 1990, now patented, Pat. No. US 5202422 which is a continuation-in-part of Ser. No. US 1989-427765, filed on 27 Oct 1989, now abandoned
DT Utility
FS Granted
EXNAM Primary Examiner: Moody, Patricia R.
LREP Logan, April C.
CLMN Number of Claims: 11
ECL Exemplary Claim: 1
DRWN 12 Drawing Figure(s); 10 Drawing Page(s)
LN.CNT 3503
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention contemplates a transgenic plant having somatic and germ cells containing at least two mammalian genes coding for polypeptides capable of autogenously associating with each other to form a biologically active multimer. In addition, the invention describes a method for producing a glycopolypeptide multimer by introducing first and second mammalian genes encoding the constituent parts of the multimer into first and second respective members of a plant species, generating a progeny from the first and second plant species members, and isolating the glycopolypeptide multimer from the progeny plant.

L22 ANSWER 13 OF 22 MEDLINE DUPLICATE 4
AN 97432065 MEDLINE
DN 97432065 PubMed ID: 9286069
TI Non-cultivable phytopathogenic mycoplasmas: characterization, detection and perspectives for control.
AU Garnier M
CS Laboratoire de Biologie Cellulaire et Moleculaire INRA BP 81, Villenave d'Ornon, France.
SO WIENER KLINISCHE WOCHENSCHRIFT, (1997 Aug 8) 109 (14-15) 613-7. Ref: 46
Journal code: XOP; 21620870R. ISSN: 0043-5325.
CY Austria
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199710
ED Entered STN: 19971105
Last Updated on STN: 19971105
Entered Medline: 19971023
AB Phytoplasmas (ex MLOs) and spiroplasmas are important groups of plant pathogenic mollicutes, discovered in 1967 and 1970 respectively. Spiroplasmas, like other mollicutes, can be cultured in artificial media and are thus well characterized. On the contrary, phytoplasmas have resisted in vitro cultivation and their study was difficult until the recent development of molecular techniques. From the sequence of their 16S rDNA, phytoplasmas have been shown to be true mollicutes. Fourteen phytoplasma subclasses have been defined, but only two phytoplasmas have so far been named at the genus and species level. Monoclonal antibodies, DNA probes and PCR primers for the specific detection of various phytoplasmas have been obtained. These showed that a given phytoplasma can infect a broad range of plants, while others are restricted to a single plant species. Specific reagents are also used for identification of insect vectors and reservoir plants of the various phytoplasmas. Plant pathogenic mollicutes cannot be controlled chemically today, since the use of antibiotic treatment is forbidden in agriculture. However, the growth and metabolism of mollicutes are known to be inhibited by antibodies and this provides a hopeful approach for future control of these agents in plants. Indeed, it has been shown recently that plants can be engineered to express and assemble functional ***immunoglobulin*** chains. Transgenic tobacco ***plants*** expressing an antibody against the stolbur phytoplasmas have been developed. They have now to be challenged with the phytoplasma to determine if they have acquired resistance to this mollicute.

L22 ANSWER 14 OF 22 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
AN 1996-333987 [33] WPIDS
DNN N1996-281425 DNC C1996-105533
TI ***Immunoglobulin*** and protection protein complex and its prodn. in ***plants*** - useful for passive immunisation against mucosal antigens,
esp. against S. mutans and S. sorbinus to prevent dental caries.
DC B04 D16 P13
IN HIATT, A C; MA, J K; LEHNER, T; MA, J K C; MOSTOV, K E; MA, J K -
PA (PLAN-N) PLANT BIOTECHNOLOGY INC; (UNME-N) UNITED MEDICAL & DENTAL SCHOOLS GUYS; (PLAN-N) PLANET BIOTECHNOLOGY INC; (HIAT-I) HIATT A C; (LEHN-I)

LEHNER T; (MAJK-I) MA J K -; (MOST-I) MOSTOV K E

CYC 33

PI WO 9621012 A1 19960711 (199633)* EN 152p
RW: AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE
W: AU BR CA CN CZ FI HU JP KR MX NO NZ PL RU SG
AU 9646088 A 19960724 (199644)
EP 807173 A1 19971119 (199751) EN
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE
JP 11504901 W 19990511 (199929) 131p
US 6046037 A 20000404 (200024)
AU 722668 B 20000810 (200043)
AU 2000071534 A 20010208 (200113)#
US 6303341 B1 20011016 (200164)

ADT WO 9621012 A1 WO 1995-US16889 19951227; AU 9646088 A AU 1996-46088 19951227; EP 807173 A1 EP 1995-944237 19951227, WO 1995-US16889 19951227; JP 11504901 W WO 1995-US16889 19951227, JP 1996-521124 19951227; US 6046037 A CIP of US 1994-367395 19941230, US 1995-434000 19950504; AU 722668 B AU 1996-46088 19951227; AU 2000071534 A Div ex AU 1996-46088 19951227, AU 2000-71534 20001110; US 6303341 B1 CIP of US 1994-367395 19941230, Cont of US 1995-434000 19950504, US 1999-312157 19990514

FDT AU 9646088 A Based on WO 9621012; EP 807173 A1 Based on WO 9621012; JP 11504901 W Based on WO 9621012; AU 722668 B Previous Publ. AU 9646088, Based on WO 9621012; AU 2000071534 A Div ex AU 722668; US 6303341 B1 Cont of US 6046037

PRAI US 1995-434000 19950504; US 1994-367395 19941230; AU 2000-71534 20001110; US 1999-312157 19990514

AB WO 9621012 A UPAB: 19960823

Immunoglobulin (Ig) comprising a protection protein (PP) in association with an Ig derived heavy chain having at least a portion of an antigen binding domain, is new. Also claimed are: (1) eukaryotic cell (pref. an alfalfa or tobacco cell) contg. (a) the claimed Ig, (b) a nucleotide sequence encoding a PP or (c) a PP; (2) plant cell contg. a nucleotide sequence encoding a PP and a Ig derived heavy chain having at least a portion of an antigen binding domain; (3) compsn. comprising the claimed Ig, and plant macromolecules; and (4) tetratransgenic organism comprised of cells contg. 4 different transgenes each encoding a different polypeptide of a multiple mol., where at least 1 of each of the different polypeptides is associated together in the multiple mol..

USE - The Ig mols. are useful for passively immunising animals against mucosal pathogens. Specifically, where the antigen binding domain is derived from the Guy's 13 antibody, the Ig can be used to prevent dental caries by binding, e.g. S. mutans serotypes c, e and f, or S. sorbinus serotypes d and g (claimed). The Ig can be administered as part of a plant extract as in (3), after manipulating taste and texture to enable oral, dental or gastric admin.

ADVANTAGE - The protection proteins protect the Ig in the mucosal environment, therefore enhancing its effectiveness. The tetratransgenic plants can efficiently assemble a tetrameric complex of alpha, J and kappa Ig chains with a specific PP.

Dwg.0/1

L22 ANSWER 15 OF 22 MEDLINE DUPLICATE 5

AN 94291711 MEDLINE

DN 94291711 PubMed ID: 8020548

TI Assembly of monoclonal antibodies with IgG1 and IgA heavy chain domains in transgenic tobacco plants.

AU Ma J K; Lehner T; Stabila P; Fux C I; Hiatt A

CS Department of Immunology, UMDS Guy's Hospital, London, GB.
SO EUROPEAN JOURNAL OF IMMUNOLOGY, (1994 Jan) 24 (1) 131-8.
Journal code: EN5; 1273201. ISSN: 0014-2980.
CY GERMANY: Germany, Federal Republic of
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199408
ED Entered STN: 19940815
Last Updated on STN: 19940815
Entered Medline: 19940802
AB The genes encoding the heavy and light chains of a murine monoclonal antibody (mAb Guy's 13) have been cloned and expressed in Nicotiana tabacum. Transgenic plants have been regenerated that secrete full-length Guy's 13 antibody. By manipulation of the heavy chain gene sequence, constant region domains from an ***immunoglobulin*** alpha heavy chain have been introduced, and ***plants*** secreting Guy's 13 mAb with chimeric gamma/alpha heavy chains have also been produced. For each plant antibody, light and heavy chains have been detected by Western blot analysis and the fidelity of assembly confirmed by demonstrating that the antibody is fully functional, by antigen binding studies. Furthermore, the plant antibodies retained the ability to aggregate streptococci, which confirms that the bivalent antigen-binding capacity of the full length antibodies is intact. The results demonstrate that IgA as well as IgG class antibodies can be assembled correctly in tobacco plants and suggest that transgenic plants may be suitable for high-level expression of more complex genetically engineered immunoglobulin molecules. Since mAb Guy's 13 prevents streptococcal colonization in humans, transgenic plant technology may have therapeutic applications.

L22 ANSWER 16 OF 22 USPATFULL
AN 93:29299 USPATFULL
TI Compositions containing plant-produced glycopolypeptide multimers, multimeric proteins and method of their use
IN Hiatt, Andrew C., San Diego, CA, United States
Hein, Mich B., Fallbrook, CA, United States
PA The Scripps Research Institute, La Jolla, CA, United States (U.S. corporation)
PI US 5202422 19930413
AI US 1990-591823 19901002 (7)
RLI Continuation-in-part of Ser. No. US 1989-427765, filed on 27 Oct 1989
DT Utility
FS Granted
EXNAM Primary Examiner: Lacey, David L.; Assistant Examiner: Budens, Robert D.
LREP Bingham, Douglas A., Fitting, Thomas, Logan, April C.
CLMN Number of Claims: 5
ECL Exemplary Claim: 1,5
DRWN 12 Drawing Figure(s); 12 Drawing Page(s)
LN.CNT 3337
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention contemplates glycopolypeptide multimers having a polypeptide that contain an immunoglobulin amino acid residue sequence and an oligosaccharide that comprises a core pentasaccharide and N-acetylglucosamine-containing outer branches, such that the multimer is free from sialic acid. The production of passive immunity in an animal by administering a sialic acid free glycopolypeptide multimer is also contemplated. In addition, the invention describes a method for

producing a glycopolypeptide multimer by introducing first and second mammalian genes encoding the constituent parts of the multimer into first and second respective members of a plant species, generating a progeny from the first and second plant species members, and isolating the glycopolypeptide multimer from the progeny plant.

L22 ANSWER 17 OF 22 MEDLINE DUPLICATE 6
AN 92003696 MEDLINE
DN 92003696 PubMed ID: 1717050
TI 'Phytoantibodies': a general vector for the expression of ***immunoglobulin*** domains in transgenic ***plants*** .
AU Benvenuto E; Ordas R J; Tavazza R; Ancora G; Biocca S; Cattaneo A; Galeffi P
CS ENEA Dipartimento Ricera e Sviluppo Agroindustrali, Divisione Ingegneria Genetica C.P.2400, Roma, Italy.
SO PLANT MOLECULAR BIOLOGY, (1991 Oct) 17 (4) 865-74.
Journal code: A6O; 9106343. ISSN: 0167-4412.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199111
ED Entered STN: 19920124
Last Updated on STN: 19960129
Entered Medline: 19911121
AB Sequences encoding the immunoglobulin heavy-chain variable (VH) domains were engineered in a new general purpose vector to transform plants via Agrobacterium. The expression of an isolated VH domain (IVD) after introduction into the plant genome has been monitored by northern, western and immunohistochemical analysis. Immunoblotting showed that the polypeptide was stably expressed and accounted for up to 1% of the soluble protein fraction. It is therefore proposed that single ***immunoglobulin*** domains of suitable specificity expressed in ***plants*** may constitute an effective system to inhibit the activity of molecules involved in plant pathology or plant development.

L22 ANSWER 18 OF 22 MEDLINE DUPLICATE 7
AN 91199725 MEDLINE
DN 91199725 PubMed ID: 1707780
TI Opportunities for bioactive compounds in transgenic plants.
AU Hall T C; Bustos M M; Anthony J L; Yang L J; Domoney C; Casey R
CS Biology Department, Texas A&M University, College Station 77843-3258.
SO CIBA FOUNDATION SYMPOSIUM, (1990) 154 177-94; discussion 194-7. Ref: 86
Journal code: D7X; 0356636. ISSN: 0300-5208.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 199105
ED Entered STN: 19910607
Last Updated on STN: 20000303
Entered Medline: 19910521
AB A variety of bioactive compounds have now been introduced into plants through recombinant DNA techniques. Early examples included genes encoding proteins conferring herbicide tolerance and insect or virus resistance.

More recently, pharmacologically useful compounds such as enkephalin and ***immunoglobulin*** have been produced in transgenic ***plants*** . Modification of existing compounds to provide better nutritional value or improved functional properties is exemplified in the case of seed storage proteins. The value of RNAs as bioactive compounds for suppression of undesirable products and viral infection has now been demonstrated in plants. The developmentally regulated expression of novel bioactive compounds in defined tissues, and their targeting to specific subcellular locations, is becoming of ever increasing economic and sociological importance as knowledge of the molecular mechanisms involved accumulates.

L22 ANSWER 19 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1988:379232 BIOSIS
DN BA86:63142
TI ISOLATION PURIFICATION AND SEROLOGY OF RICE TUNGRO BACILLIFORM AND RICE TUNGRO SPHERICAL VIRUSES.
AU CABAUATAN P Q; HIBINO H
CS INT. RICE RES. INST., P.O. BOX 933, MANILA, PHILIPPINES.
SO PLANT DIS, (1988) 72 (6), 526-528.
CODEN: PLDIDE. ISSN: 0191-2917.
FS BA; OLD
LA English
AB Rice [Oryza sativa L.] seedlings were inoculated by rice green leafhoppers (Nephotettix virescens) that had fed on rice plants infected with both rice tungro bacilliform virus (RTBV) and rice tungro spherical virus-infected plants were identified and selected using antiserum to rice waika virus which is very closely related, if not identical to, RTSV. Rice tungro spherical virus was propagated by inoculating rice seedlings using leafhoppers. To multiply RTBV, seedlings were inoculated by leafhoppers that had fed first on plants infected with both RTBV and RTSV, second on anti-RTSV ***immunoglobulin*** through membrane, and then on RTBV-infected ***plants*** . Rice tungro bacilliform virus and RTSV were purified separately from their respectively infected plants by heating sap 1 hr at 40 C, by driselase treatment, and by polyethylene glycol precipitation, differential centrifugations, and sucrose density gradient centrifugation. Purified RTBV fractions contained bacilliform particles 30-35 nm in width and 160-220 nm in length. Purified RTSV fractions contained isometric particles 30 nm in diameter. Both fractions had UV absorption spectra typical of nucleoprotein. Rabbit antisera obtained had titers of 1/2,560 for RTBV and 1/640 for RTSV by the ring-interface precipitin test. The latex test and ELISA specifically detected RTBV and RTSV in leaf extracts. The antisera were virus-specific.

L22 ANSWER 20 OF 22 WPIDS COPYRIGHT 2002 DERWENT INFORMATION LTD
AN 1985-007635 [02] WPIDS
DNC C1985-003107
TI Capsule with crosslinked protein walls around living cells - useful as source of antibodies, etc. e.g. injection.
DC A96 B04 D16
IN MEYERS, W E; TICE, T R
PA (STOL-N) STOLLE RES & DEV
CYC 14
PI EP 129619 A 19850102 (198502)* EN 28p
R: AT BE CH DE FR GB IT LI LU NL SE
JP 60025929 A 19850208 (198512)#
CA 1214389 A 19861125 (198652)#
EP 129619 B 19880518 (198820) EN

R: AT BE CH DE FR GB IT LI LU NL SE
DE 3376660 G 19880623 (198826)
JP 04008034 B 19920213 (199211)# 8p
JP 05176754 A 19930720 (199333)# 7p
JP 06085711 B2 19941102 (199442)#
ADT EP 129619 A EP 1983-303605 19830622; JP 04008034 B JP 1983-131097 19830720; JP 05176754 A Div ex JP 1983-131097 19830720, JP 1991-287274 19830720; JP 06085711 B2 Div ex JP 1983-131097 19830720, JP 1991-287274 19830720
FDT JP 06085711 B2 Based on JP 05176754
PRAI EP 1983-303605 19830622
AB EP 129619 A UPAB: 19941122
Capsule contg. living cells and having a wall comprising a cross-linked protein is new.

Pref. the protein is albumin, casein, collagen, gelatin, soy protein, gluten or immunoglobulin. The wall has pores of 5 Angstroms to 15 micrometres. There may be an approp. nutrient medium for the cells in the capsules. Suitably they have average dia. less than 250 micrometres.

USE/ADVANTAGE - The living cells can be encapsulated under sufficiently mild conditions for them to retain viability while a controlled porosity can be formed in the capsule walls. The cells may be used as a source of macromolecules or biological prods., such as antibodies or virions, and such macromolecules can pass through the pores in the capsule wall. Similarly when the capsules are injected into a host, while the entry of host cells into the capsules to destroy the cells is prevented.

L22 ANSWER 21 OF 22 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1983:240854 BIOSIS
DN BA75:90854
TI A HYDROXY PROLINE-RICH BACTERIAL AGGLUTININ FROM POTATO SOLANUM-TUBEROSUM CULTIVAR KATAHDIN ITS LOCALIZATION BY IMMUNO FLUORESCENCE.
AU LEACH J E; CANTRELL M A; SEQUEIRA L
CS DEP. PLANT PATHOL., UNIV. WISCONSIN, MADISON 53706, USA.
SO PHYSIOL PLANT PATHOL, (1982 (RECD 1983)) 21 (3), 319-326.
CODEN: PPPYBC. ISSN: 0048-4059.
FS BA; OLD
LA English
AB Potato tubers (cv. Katahdin) contain a hydroxyproline-rich glycoprotein (HPRG) that agglutinates certain avirulent strains of the bacterial wilt pathogen, Pseudomonas solanacearum. This and similar agglutinins are thought to play an important role in the immobilization of incompatible bacteria in potato and tobacco tissues. The agglutinin from potato tubers was purified by ion exchange chromatography Antisera to the intact or deglycosylated agglutinin were obtained from New Zealand white rabbits after multiple intradermal and intramuscular injections. Immunoglobulins were precipitated with (NH4)2SO4 and antibodies specific for the agglutinin were purified by affinity chromatography. Frozen sections of petiole or leaf tissue from tobacco and potato were treated firstly with sheep normal immunoglobulin and then with either anti-agglutinin antibodies or normal rabbit immunoglobulin for 20 min. The sections were rinsed and then treated with fluorescein isothiocyanate-conjugated sheep anti-rabbit immunoglobulin. When the sections were examined by fluorescence microscopy, it was determined that anti-agglutinin antibodies bound only to the cell walls, particularly those of parenchyma. Fluorescence was also evident on the cell walls of tobacco and potato xylem vessels, epidermis and collenchyma. Control sections treated with

normal rabbit immunoglobulin did not bind the labeled anti-rabbit ***immunoglobulin*** . Cell walls in tissue sections from non-solanaceous ***plants*** such as soybean, corn or begonia, treated in the same manner, were also stained by the labeled antibodies. Antibodies to both intact and deglycosylated potato agglutinin bound to these plant cell walls, indicating that the receptors are proteins with antigen determinants which are similar to those of proteins from potato or tobacco cell walls. Such proteins (HPRGs) are common components of plant cell walls and may play a role in immobilizing bacteria that gain access to the intercellular spaces.

L22 ANSWER 22 OF 22 MEDLINE DUPLICATE 8
AN 76252563 MEDLINE
DN 76252563 PubMed ID: 821467
TI Identification of N-terminal methionine in the precursor of ***immunoglobulin*** light chain. Initiation of translation of messenger ribonucleic acid in ***plants*** and animals.
AU Schechter I; Burstein Y
SO BIOCHEMICAL JOURNAL, (1976 Mar 1) 153 (3) 543-50.
Journal code: 9YO; 2984726R. ISSN: 0264-6021.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 197609
ED Entered STN: 19900313
Last Updated on STN: 19900313
Entered Medline: 19760925
AB The proteins programmed in the wheat-germ cell-free system by the mRNA coding for the MOPC-321 mouse myeloma L (light) chain were labelled with [35S]methionine, [4,5-3H]leucine or [3-3H]serine, and were subjected to amino acid-sequence analyses. Over 95% of the total cell-free product was sequenced as one homogeneous protein, which corresponds to the precursor of the L-chain protein. In the precursor, 20 amino acid residues precede the N-terminus of the mature protein. This extra piece contains one methionine residue at the N-terminus, one serine residue at position 18, and six leucine residues, which are clustered in two triplets at positions 6, 7, 8 and 11, 12, 13. The identification of methionine at the N-terminus of the precursor is in agreement with the evidence showing that unblocked methionine is the initiator residue for protein synthesis in eukaryotes. The absence of methionine at position 20, which precedes the N-terminal residue of the mature protein, suggests that myeloma cells synthesize the precursor. However, within the cell the precursor should be rapidly processed to the mature L chain, since precursor molecules have not yet been found in the intact animal. The abundance (30%) of leucine residues indicates that the extra-piece moiety is quite hydrophobic. The extra piece of the MOPC-321 L-chain precursor synthesized with the aid of the Krebs II ascites cell-free system is of identical size and it has the same leucine sequence [Schechter et al. (1975) Science 188, 160-162]. This indicates that cell-free systems derived from the plant and animal kingdom initiate mRNA translation from the same point. It is shown that the amino acid sequence of minute amounts of a highly labelled protein (0.1 pmol) can be faithfully determined in the presence of a large excess (over 2000 000-fold) of unrelated non-radioactive proteins.

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=> s (tumo!r specific vaccine)(20w)(plant)
L23           0 (TUMO!R SPECIFIC VACCINE)(20W)(PLANT)

=> s (tumo!r specific vaccine)(20w)(transformed plant)
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S nt: Wednesday, April 03, 2002 3:48 PM
To: STIC-ILL
Subject: 09522900

Please provide copies of

1. Hakim et al 1996 j of Immunology vol 157, pgs 5503-5511.

2. Carroll et al 1988 J Exp. MEd vol 168, pgs 1607-1620.

Thanks
Geetha BansalAU 1642
305-3955
CMI/8A03
AU 1642

ALTERNATIVE V_K GENE REARRANGEMENTS IN A MURINE B CELL LYMPHOMA

An Explanation for Idiotypic Heterogeneity

By WILLIAM L. CARROLL, CHARLIE O. STARNES, RONALD LEVY, AND SHOSHANA LEVY

From the Department of Medicine, Stanford University School of Medicine, Stanford, California 94305

The diversity of the antigen binding portion of the Ig molecule is generated through the recombination of multiple discontinuous gene segments (1, 2). The heavy chain variable (V) region coding sequence is formed when one of hundreds of V_H regions combines with one of 15–20 D regions and one of 4 J_H regions to create a complete VDJ segment (3, 4). Light chain recombination proceeds in a similar fashion between V_L and J_L segments (5). These events occur in a well-defined order with DJ_H rearrangement preceding V_H-to-DJ_H recombination (6, 7), followed later by V_L-to-J_L recombination. The exact site of recombination between these segments is imprecise, thus creating further diversity at the joints. Single nucleotides (N segments) may be added at the limits of the D region in heavy chain rearrangements (8, 9). These mechanisms, along with the association of different heavy and light chains, result in an expected antibody repertoire of $>10^{11}$ Ig molecules.

In recent years it has become apparent that rearranged VDJ segments are capable of undergoing further somatic variation. A major mechanism of generating further diversity is somatic point mutation within the V region coding sequence (1, 10). This process appears to be focused within and nearby the rearranged V region genes. Somatic point mutation of these genes has been estimated to occur at a rate as high as 10^{-3}/bp/generation (11, 12). It occurs during the late primary and during the generation of the secondary immune response (13). It may alter both antigen specificity and affinity (14). In addition to somatic mutation, less frequently implicated molecular events such as gene conversion or V_H region replacement may modify already expressed *Ig* genes (15–18). These processes appear to occur during specific stages of B cell differentiation and may be limited to certain B cell subsets (17).

The molecular basis of somatic diversification may be studied in greater detail by examining B cell tumors. Tumors that represent discrete stages or lineages of normal B cell development and have been adapted to growth in vitro should be good

This work was supported by National Institutes of Health grants CA-34233 and CA-33399 from the U. S. Public Health Services. R. Levy is an American Cancer Society Research Professor. W. L. Carroll was a recipient of NIH Clinical Investigator Award CA-01059 and C. O. Starnes was supported by U. S. Public Health Service grant AI-07290. Computer resources used to carry out this study were provided by the NIH-sponsored BIONET National Computer Research for Molecular Biology (grant RR-01685-05). Address correspondence to S. Levy, Dept. of Medicine, Stanford University School of Medicine, Stanford, CA 94305. W. Carroll's present address is Dept. of Pediatrics/Hematology-Oncology, Washington University School of Medicine, St. Louis, MO 63110.

candidates f r such studies. Recently we have described surface Ig^+ idiotype-negative (sIg^+, Id^-)[1] tumor variants in the murine lymphoma 38C13 (19). This B cell lymph ma bears surface Igκ but secretes little of this molecule, and therefore, is an excellent model for immunotherapy targeted against its Id. We generated a panel of syngeneic antiidiotype mAbs and have examined their antitumor effects (20, 21). We found minor tumor subpopulations that failed to react with one or another of the antiidiotype antibodies. Such variants could be selected either in vivo (19) or in vitro. Previously we reported biochemical and serologic differences between tumor variants (19). Antisera and mAbs were produced that distinguished each individual variant Id protein. The Ig produced by the tumor variants differed from one another in the migration of their κ light chain proteins on SDS-PAGE. We found that their κ light chain gene rearrangements were also different from one another and from the parental 38C13 lymphoma. Here we describe the molecular basis of this heterogeneity.

Materials and Methods

Tumor Cell Lines. The 38C13 murine B cell lymphoma of C_3H origin, and mAbs directed against its cell surface idiotypic determinants have been described in detail previously (20–23). Tumor cell lines were grown in RPMI 1640 medium (Irvine Scientific, Irvine, CA), supplemented with 12% FCS, penicillin, streptomycin (Sigma Chemical Co., St. Louis, MO), L-glutamine (Gibco Laboratories, Grand Island, NY), 10 mm Hepes (Gibco Laboratories), and 5×10^{-5} M 2-ME in a humidified 37°C, 5% CO_2 incubator. Id variants were selected by methods outlined earlier (19). Briefly, four different classes of tumor variants were isolated on the basis of surface Ig expression and binding to two different anti-Id antibodies, S1C5 and S5A8. Three of these variants were recovered from mice whose tumors regrew after therapy in vivo with S1C5. One of these variants, V1, retained surface Ig expression, but failed to bind both anti-Id mAbs. Variant V2 lost surface Ig expression altogether. Another variant, V3, was sIg^+, lost binding to antibody S1C5, but still bound antibody S5A8. A fourth variant, V4, was isolated after the 38C13 tumor was exposed in vitro to S5A8 coupled to recombinant A chain of ricin. This sIg^+ variant lost binding to antibody S5A8 but retained binding to S1C5. Binding in all these cases was assessed on whole cells by flow cytometry and confirmed by ELISA on Ig proteins isolated from the variant tumor cells (see below). These characteristics are summarized in Table I.

To recover large amounts of protein and mRNA for cDNA synthesis, variant tumor cell lines were fused to the nonsecreting mouse myeloma 8.653 as previously described (19, 23). Hybrids were selected with HAT medium. The absence of 2-ME prevented the growth of parental tumor cells. The resulting hybrids were screened for Ig production and were cloned by limiting dilution. Protein was isolated by affinity chromatography and anti-Id binding was examined by the ELISA method (19). In each case the Id determinants expressed on the tumor cell surface and by the isolated protein were identical.

Southern Analysis of Rearranged V_κ Genes. High molecular weight DNA was isolated from the original 38C13 lymphoma, the tumor variants V1-V4, their respective hybridomas, the P3x63 8.653 myeloma fusion partner, and C3H liver. 10 μg of DNA was digested with Bam HI restriction endonuclease (New England Biolabs, Beverly, MA) and loaded on a 0.8% agarose gel. Samples were transferred to an activated nylon filter (Genatran 45; Plasco Inc., Woburn, MA) according to the method of Southern (24). Filters were baked under vacuum at 80°C for 2 h. Prehybridization was carried out at 42°C for 6 h in 50% formamide, 3× SSC, 5× Denhardt's solution, 1 mM sodium pyrophosphate, and 100 μg/ml of denatured salmon sperm DNA.

Hybridization was performed under identical conditions with the exception of the addi-

[1] *Abbreviations used in this paper:* Id, idiotype; sIg, surface immunoglobulin.

TABLE I
mAb Reactivity of Tumor Subclones

Tumor	mAb		
	ακ	S1C5*	S5A8*
38C13	+	+	+
V1	+	–	–
V2	–	–	–
V3	+	–	+
V4	+	+	–

* Syngeneic antiidiotype mAbs raised against the 38C13 Ig protein.

tion of 8% Dextran sulphate and the radioactive probe. The filter was washed in 2× SSC 0.1% SDS at room temperature for 1 h followed by a more stringent wash in 0.1 × SSC/0.1% SDS at 62°C for an additional hour. Two probes were used to analyze κ rearrangements: a 500-bp Eco RI–Hpa I constant region cDNA clone and a Hind III 2.8-kb genomic clone encompassing the entire germline J_κ region (5). Probes were labeled to high specific activity using the random primer method (25).

Northern Analysis of TdT Expression. Poly(A)$^+$ mRNA from 38C13 and from EL-4 cells (a gift from D. Daney, Stanford University) was electrophoresed on a formaldehyde-agarose gel and transferred to a nitrocellulose filter as described (26). The filter was baked, prehybridized, hybridized, and washed as described for Southern hybridization. The mouse TdT probe (27) was a 1.8-kb insert in pUC13 and was a gift from N. Landau, University of California, School of Medicine, San Francisco, CA.

Cloning and Sequencing Variant V_μ and V_κ Genes. cDNA libraries were made from each hybridoma generated from the sIg$^+$ tumor variants. Southern blot analysis of rearranged heavy and κ light chain genes showed identical rearrangements in both the parental tumor line and the representative hybridoma, which insured that fidelity of Ig gene structure was preserved through the fusion process (19). cDNA libraries were made using a highly efficient method described in detail previously (28). RNA was extracted as previously described (29) and poly(A)$^+$ RNA was selected over an oligo-dT-cellulose column (30). First strand cDNA was made using reverse transcriptase (Life Sciences, St. Petersburg, FL) and primers specific for the μ heavy chain (5′-CAGGAGACGAGGGGGAA-3′) and κ light chain (5′-TGGATGGTGGGAAGATG-3′) constant regions just 3′ to the variable region. Second strand was synthesized with DNA polymerase I (New England Biolabs) and RNase H (Bethesda Research Laboratories, Gaithersburg, MD) (31). The resulting double-stranded cDNA was polished with T4 DNA polymerase (New England Biolabs) and ligated directly into a Sma I–digested (New England Biolabs), CIAP (Boehringer Mannheim Biochemicals, Indianapolis, IN)-treated (32), m13mp19 sequencing vector (33). JM101 bacteria were made competent by the Hanahan method and were transformed with the recombinant m13 (34). The resulting plaques were lifted onto nitrocellulose filters twice and filters were baked for 2 h at 80°C under vacuum. Filters were prehybridized, hybridized, and washed as described above for Southern hybridization. Filters were dried and exposed to XAR film (Eastman Kodak Co., Rochester, NY) for 1–6 h. Positive clones were identified and single-stranded sequencing templates were made by standard techniques (33). Clones were sequenced by the dideoxy method (35). If the isolated clones were in one orientation only, m13 replicative form was isolated and the insert was cloned in the opposite orientation (using flanking restriction sites Eco RI, Bam HI) into m13mp18.

V region–specific probes for plaque screening were obtained as follows. The 38C13 μ heavy chain gene had previously been isolated from a λgt11 total cDNA tumor library (36). An Eco RI/Sma I fragment encompassing the entire V region and a portion of CH1 was used to screen and isolate μ clones from each variant cDNA library. A 550 bp Hpa I/Eco RI V_κ region (37) was used as a probe for the isolation of κ clones from the variant tumor libraries.

Results

Tumor Variants Display Heterogeneous V_κ Rearrangements and Identical Reciprocal Product. We previously reported on Southern blot analysis of the rearranged V_κ alleles in these tumor variants using a constant region probe (Fig. 1 *A*) (19). Each tumor shares an identical pattern of rearrangement with its respective hybridoma and demonstrates that Ig gene structure was preserved through the fusion process. In addition, each hybrid retains 7.2-kb and 5.2-kb rearrangements inherited from the myeloma fusion partner 8.653. Uncloned 38C13 lymphoma showed a major 8.9-kb band along with three other less prominent rearrangements (8.2, 3.7, and 3.4 kb). Each variant displayed two rearrangements, some of which were also identified in the 38C13 tumor. The pattern of rearrangement differed among the tumor variants, although in some cases they appeared to share a single rearrangement. The same filter was washed and reprobed with a genomic fragment containing the J_κ region (Fig. 1 *B*). Each of the bands detected with the C_κ probe in the tumor DNA also hybridized to the J_κ probe with the exception of the lower faint rearrangement (3.4 kb) in the 38C13 tumor. Therefore, these bands represent V/J rearrangements linked to the κ constant region segment. In addition, a larger ~15-kb band was seen with the J_κ probe in 38C13 and in each tumor variant. This band did not hybridize to the constant region probe and indicates that a portion of the J_κ sequence was retained in the genome and was displaced from its normal association with C_κ. The constancy of this reciprocal gene product provides an independent confirmation of the clonal origin of these tumor variants. The 5.2-kb band that originated from the 8.653 fusion partner did not hybridize with the J_κ probe.

All Tumor Variants Contain Identical Heavy Chain V Region. Full-length V_H clones were isolated from the V1 and the V4 variant tumor cDNA libraries. A partial sequence from the V3 variant tumor was also determined. All of these sequences are identical (Fig. 2). Further comparison of those V regions with the published 38C13 sequence (36) demonstrated some minor differences. Therefore, the original 38C13 μ V region clone was resequenced in both orientations. It was identical to the variants described here and the previously reported 38C13 heavy chain sequence is now displayed in its corrected form (Fig. 2). This V_H gene, used by the 38C13 tumor and its variants, is most homologous to the previously reported germline VB6.5 gene of the T15 family derived from a mouse of the B10.P strain (38). The two genes differ from each other in three positions. These differences might be explained by a germline difference between the C3H and the B10.P mice or by somatic mutations of the expressed 38C13 V_H gene. However, since the parental V_H sequence and those of all the variants, which have been separated by years in culture, are identical, there is no evidence of somatic mutation in the tumor.

Thus, the original 38C13 tumor and the three sIg^+ variants are all clonally related. They use the same heavy chain V gene and have identical V/D and D/J joints. In addition, the V_HDJ sequences are all identical and, therefore, there is no evidence for somatic mutation of the heavy chain in this tumor. The differences in anti-Id binding in these variants (and thus the emergence of antibody resistant tumor subclones) are not explained by differences in the heavy chain. These findings corroborate the protein and *Ig* gene rearrangement analysis that we reported previously (19).

All Tumor Variants Use a κ Light Chain Gene Different from the Original 38C13. Full-

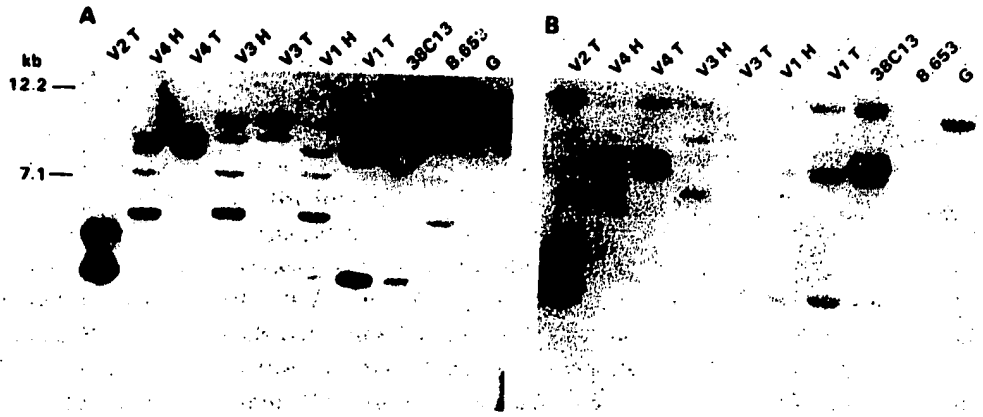

FIGURE 1. Southern blot analysis of L chain gene rearrangements. DNA was isolated from the 38C13 tumor, variant tumors (*T*) and respective hybridomas (*H*). The DNA was digested with Bam HI and probed with C_κ (*A*) and J_κ (*B*).

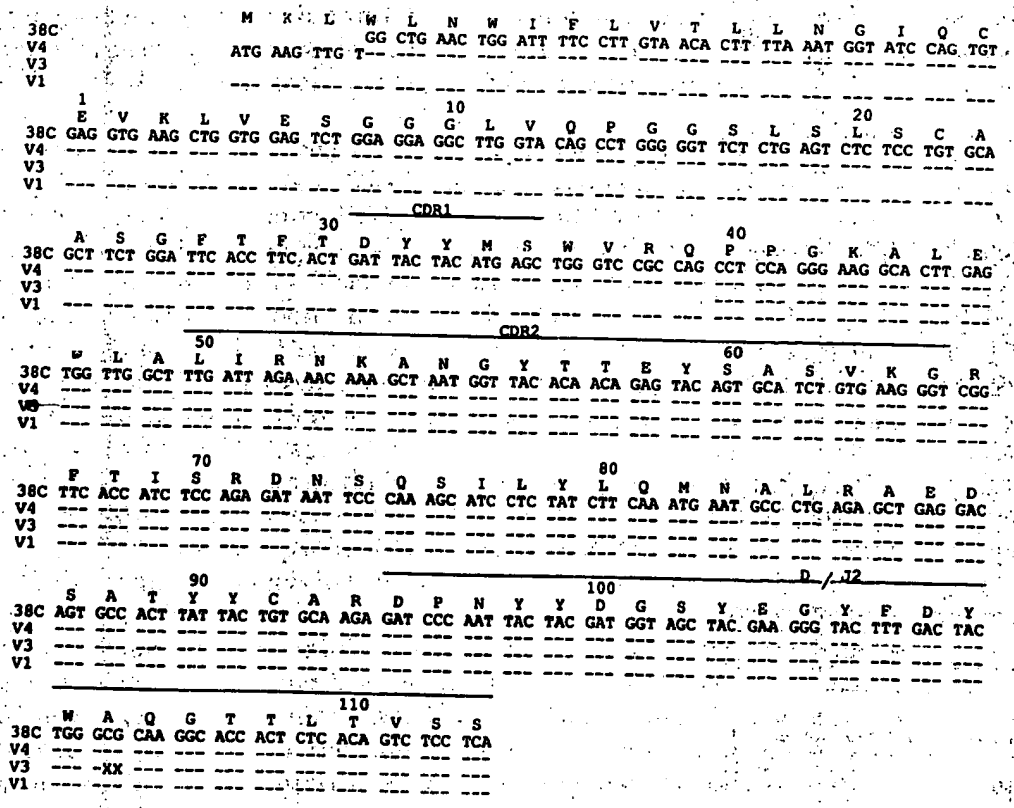

FIGURE 2. Nucleotide sequences of V_H DNA. The amino acid translation is given above the nucleotide sequences and numbered according to Kabat et al. (42). The complementarity-determining regions CDR1 and CDR2 and the D and J segments are indicated. X, ambiguous bases.

length κ V region clones were isolated from all sIg$^+$ tumor variants. The nucleic acid sequences are shown in Fig. 3 and the translated amino acid sequence is displayed in Fig. 4. All the v_κ genes expressed by the variants were similar to one another and appear to be representatives of the highly complex V_κ-Ox1 family (39). In fact, the sequence of V1 differs by only a single nucleotide from the previously reported BALB/C germline gene, R2 (39). Moreover the two κ light chain genes from tumor variants V3 and V4 were identical to each other in their V segments and their leader sequences. This V region gene differed by only five nucleotides from the previously reported NQ10.4.61 antiphenyloxazolone-producing hybridoma, de-

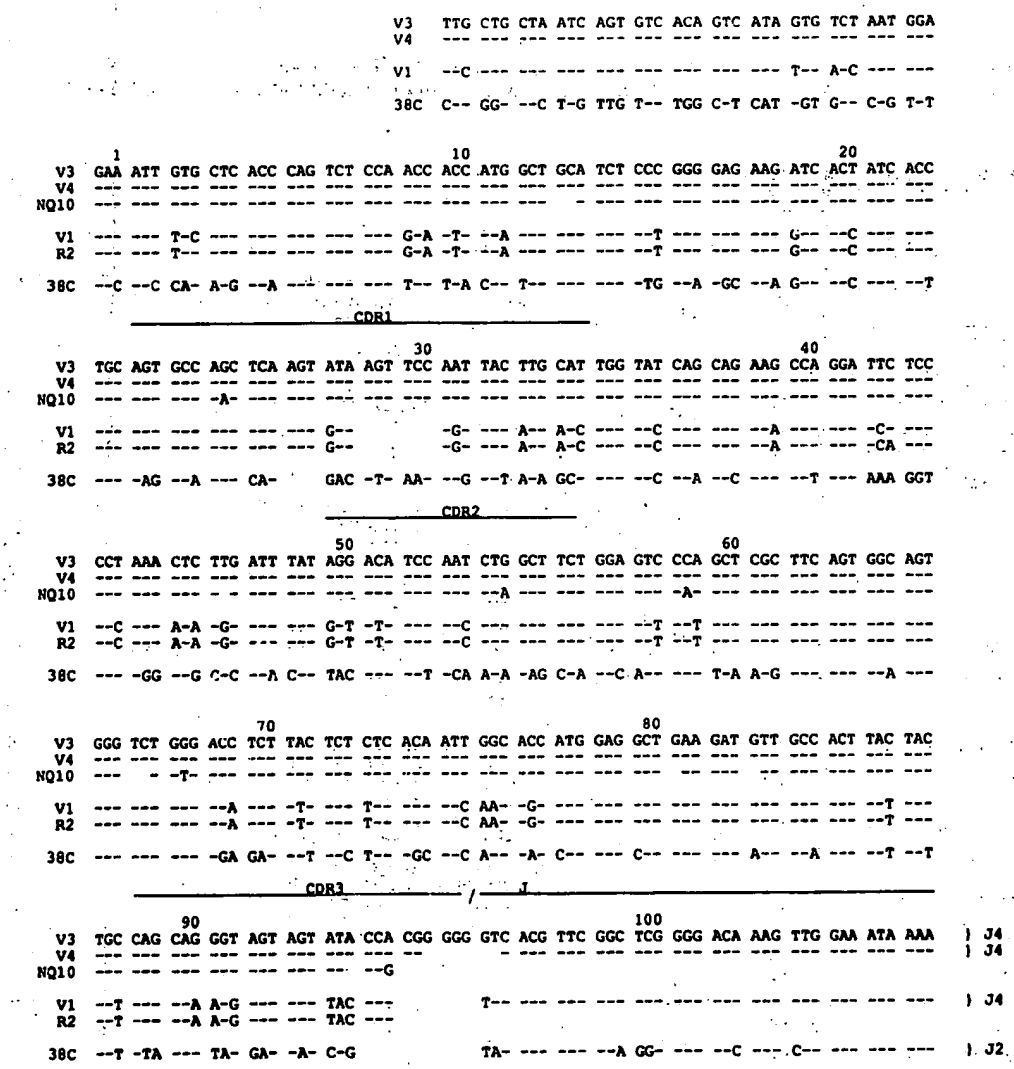

FIGURE 3. Nucleotide sequences of V_κ DNA. The nucleotides are grouped in codons and numbered according to Kabat et al. (42). The CDRs are indicated, as well as the J regions used by the 38C13 tumor (38C) and its variants. R2 and NQ10 (NQ10.4.61) are previously reported germline and mRNA sequences, respectively (39), and are both members of the V_κ-Ox1 family.

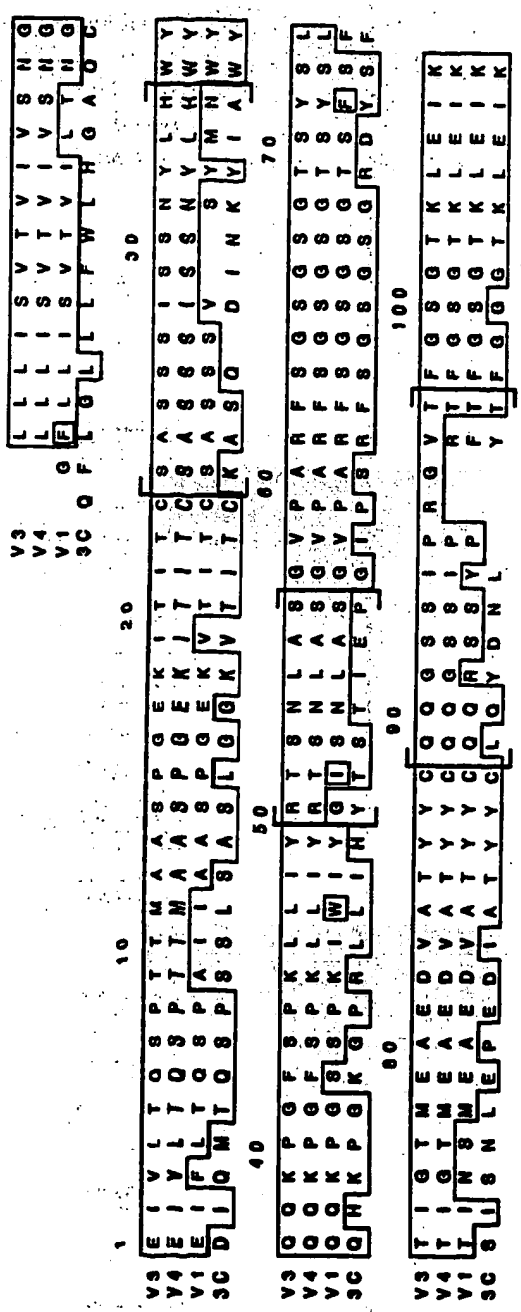

FIGURE 4. Amino acid translation of the V_K tumor variants and the 38C13 tumor (3C).

rived from another member of the V_κ-Ox1 gene family (39). By comparison, the parental 38C13 tumor expressed a completely different V_κ gene, a member of the V_κ9 family (40). All of the variants used J_κ4. By comparison, the parental 38C13 tumor use J_κ2.

Close inspection of the third hypervariable region reveals considerable variation in length among variants and 38C13. This is highly unusual since κ CDR3s tend to be constant in length (41), in contrast to the variation in length of the CDR3 region of Ig heavy chains (42). Fig. 3 illustrates the contribution of V region and J_κ segments to CDR3. In V3 and V4 there are additional GC nucleotides present at the V/J boundary. Such nucleotides are highly unusual for κ light chains and have only rarely been reported previously (41, 43, 44). Since tumors V3 and V4 share identical heavy chain sequences and V_κ regions, and both rearrange to J4, the variable addition of these extra nucleotides at the exact V/J recombination site is responsible for their different pattern of anti-Id binding.

38C13 Expresses Terminal Transferase. Poly(A)$^+$ mRNA from 38C13 cells and from EL-4 cells, which are known to express TdT, were electrophoresed on a formaldehyde-agarose gel and transferred to a nitrocellulose filter. The filter was probed with the mouse TdT probe (Fig. 5). Lanes *1* and *2* contain 0.5 μg and 1 μg of EL-4 mRNA. Lane *3* contains 8.5 μg of 38C13 mRNA. It is evident that 38C13 cells express TdT mRNA, albeit in ~1/10 of the level expressed in the EL-4 cells.

Discussion

In a previous report we described the isolation of variant tumor cells from the 38C13 B cell lymphoma after immunoselection with antiidiotype antibodies (19). These variant tumor cells were of two major types, those that had completely lost expression of Ig and those that continued the expression of Ig with an altered structure. Analysis of the Igs produced by these latter variants suggested that the μ heavy chains were similar to the wild-type tumor but that the κ chains were different in apparent molecular weight. Analysis of the *Ig* gene rearrangements in these cells suggested that the heavy chain genes expressed in these cells were identical but that the light chain genes were different. A number of possible explanations were considered for these differences in light chain structure. Among these were the possibility

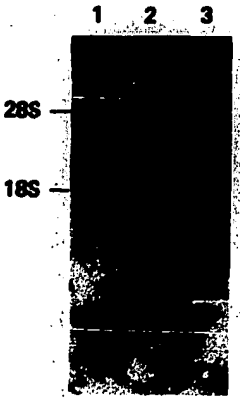

FIGURE 5. Northern blot analysis of TdT expression. mRNA from EL-4 and 38C13 cells were electrophoresed on a formaldehyde-agarose gel and transferred to nitrocellulose filter. The filter was probed with a mouse TdT probe (27). (Lanes *1* and *2*) 0.5 μg and 1.0 μg of EL-4 mRNA, (lane *3*) 8.5 μg of 38C13 mRNA. Markers indicate migration of 28S and 18S RNA.

of somatic point mutation, and continuing or alternative rearrangements at the light chain l cus.

In the present report, we directly analyzed the molecular basis of the Ig heterogeneity in these tumor variants. cDNA was cloned for both the heavy chain and light chain genes from each of the variant tumor cells and the original wild-type cell population. Comparison of the heavy chain sequences showed that they were identical in all of these cells. This result establishes that the 38C13 tumor cell population is not undergoing V gene somatic mutation. In addition, the results indicate that the B cell that gave rise to the tumor had not mutated its heavy chain gene to any significant extent because it differed by only three nucleotides from a known germline V_H gene VB6.5 (38). Similar arguments can be made from the lack of somatic mutation in the light chain genes. The identity of the V_H sequences confirmed the previous suggestions from protein and Southern blot analysis that these tumor cells were all derived from a single original cell, that is that they are clonally related. Further confirmation of the clonality of the cell populations comes from analysis of light chain gene rearrangements using the J_κ region probe (Fig. 1 *B*). In addition to the bands that comigrated with those detected by the C_κ probe there was an additional band, detected with the J_κ probe, which was common to all of the variant tumor cells and to the parental cell. Rearranged fragments of DNA containing J sequences are known to be produced during the process of V/J joining when the V region used is in inverted orientation with respect to the J and constant region segments (45). Such reciprocal products of V/J joining can be retained in their original form (46).

From these data it appears that a plausable order of events in the cell that gave rise to this tumor were the rearrangement of a unique heavy chain V region gene followed by V_κ rearrangement on both alleles; either one of which could have given rise to a J_κ reciprocal product. The absence of a common C_κ band in tumor variants would then imply that subsequent rearrangements occurred at both κ alleles in progeny cells. Ongoing light chain rearrangements have been described previously in an Abelson leukemia virus–transformed B cell line. Lewis et al. (47) showed by Southern blot analysis that a rearranged κ allele can undergo further recombination. Recently an Ableson leukemia virus pre-B cell line has been described that is capable of proceeding to λ recombination (48). Future studies on the 38C13 tumor should be able to test the possibility that the recombinase system (49) may still be active in these cells and that ongoing κ light chain recombination may be occurring. If this were the case variant tumor cells should give rise to subsequent variants in culture. If so, it will be extremely interesting to determine whether the repetitive use of the V_κ-Ox1 gene family observed in the current set of data continues to occur.

Examination of the sequences of the productive V_κ genes revealed some extremely interesting phenomena. We noticed that the V region sequence of the variant V3 and the variant V4 were identical to each other and that each of these tumors had rearranged this identical V region gene to the same J4 segment. The only difference between these genes is in the length of their CDR3 region. Each of them has additional nucleotides composed mostly of Cs and Gs in the joint between the V and J segments. V3 has an unusually long CDR3 length composed of 11 amino acids. Although the addition of extra nucleotides at the V/D/J joint is commonly seen in heavy chain gene rearrangement, it has only rarely been observed in light chain genes. In general V/J recombination takes place between codon 95 of the V_κ segment and

the first cod n, 96, of the germline J_κ segment. The exact splice junction may be located in either codon, but compensation occurs so that a CDR-3 length of nine amino acids results. However, additional nucleotides may be present 3' to the germline V_κ segment and 5' to germline J_κ located just adjacent to the respective conserved heptamer recombination sequence. In murine V_κ light chains one to two such nucleotides may be present and are usually Cs, sometimes As (44). These nucleotides have been shown to participate in V/J joining occasionally. Interestingly, Kaarttinen and Makela (41) have observed that a substantial portion of the murine $V_\kappa O_\kappa$ response consists of light chains with extra long CDR-3 segments composed of 11 amino acids, similar to V3 reported here (41). They noted the presence of up to five extra nucleotides (mostly C) at the $V_\kappa J_\kappa$ joint and postulate that these are contributed by an unusually long string of germline nucleotides 3' to V_κ before the heptamer. Up to seven nucleotides would have to be postulated to contribute to the extra segments observed in V3, and here they are mostly Gs instead of the more commonly observed Cs. Alternatively, the addition of extra nucleotides, so called N regions, in heavy chains is thought to be mediated by the action of the enzyme terminal deoxynucleotide transferase (TdT) (27). This enzyme has a preference for Gs and Cs and is thought to be inactivated by the time B cells begin to rearrange their light chain genes. Recently Heller et al. (43) described nucleotides not encoded by germline V genes or J regions at the site of V_κ/J_κ recombination in antigalactan antibodies. In addition, Klobeck et al. (44) also found a GC-rich region at the V_κ/J_κ joint in an aberrant rearrangement involved in a (2:8) translocation in a Burkitt's lymphoma cell line. Our finding of the message for TdT in the 38C13 tumor cells provides an explanation for the addition of N sequences at the V_κ joints in these cells and violates the notion that TdT is absent from cells of the fully differentiated B cell stage.

Since the V_κ/J_κ joints in variant V3 and variant V4 are different, these genes must have been created by independent joining events in the different subclones of this tumor, even though the same V gene was used. Interestingly, the V gene used by these two variant tumor cells is virtually identical to a gene used by a hybridoma with a binding activity for phenyloxazolone and is a member of the V_κ-Ox1 gene family (39). Inspection of the nucleic acid sequence of variant V1 shows that a different V_κ gene has been used by this variant, but that it is virtually identical to another previously reported member of the V_κ-Ox1 gene family R2 (39). All of these genes of variants V1, V3, V4, and the two previously reported members of the V_κ-Ox1 family are completely different from the gene used by the original 38C13 tumor cell, which is a member of a different V gene family. Therefore, it appears that this tumor has chosen alternative rearrangements at the V_κ light chain locus to create a number of different subclone variants of the prototype tumor cell, and that all of these variants have been created by the use of genes from the same V region family. It is possible that the repetitive use of the same gene family for rearrangements is based on the proximity of the V_κ-Ox1 gene family to the J region. Subsequent studies on the organization of the V_κ locus as well as that of the locus in the 38C13 tumor cell should be able to provide a test of this proximity hypothesis.

These studies on the sequence of the expressed V_κ genes in variants of the 38C13 tumor have provided an explanation for the idiotypic heterogeneity of the tumor population. As previously suspected from studies on the isolated light chain proteins and on Southern blot analysis of the light chain genes, the tumor heterogeneity

at the Id level is completely accounted for by variations in the amino acid sequence of the light chains. This variation can be extremely subtle. For instance, the entire difference between variant V3 and V4 is accounted for by three amino acids at the V/J joint of the light chain. Yet these two variants are easily distinguishable by a panel of syngeneic antiidiotype mAbs. It is clear from these studies that at some time in the history of this tumor alternative light chain gene rearrangements rather than somatic point mutation occurred and created Id diversity within the tumor cell population.

Summary

Idiotype variants of 38C13, a murine B cell lymphoma, have been isolated by immunoselection with antiidiotype mAbs. The V region genes for the κ light chains and μ heavy chains expressed by these tumor cells were sequenced and compared. There was no evidence for V region somatic point mutation in this tumor. However, while the heavy chain genes were all identical, the light chain genes were all different. The light chain genes of each variant were derived from the V_κ-Ox1 gene family and joined to $J_\kappa 4$, whereas the light chain gene of the parental tumor was derived from the $V_\kappa 9$ family and joined to $J_\kappa 2$. Two of the variants used the identical V_κ gene but differed by the inclusion of a variable number of additional nucleotides in the V/J joint. Thus, the idiotypic heterogeneity of this B cell lymphoma arises as a consequence of alternative light chain rearrangements rather than point mutation. This process repetitively uses members of the same V_κ gene family. Two of the variants use the identical V_κ and J_κ gene segments but differ by the presence of extra nucleotides at the V_κ/J_κ joint.

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A Nine-Amino Acid Peptide from IL-1β Augments Antitumor Immune Responses Induced by Protein and DNA Vaccines[1]

Itzhak Hakim, Shoshana Levy,[2] and Ronald Levy

The idiotypic determinants of B cell lymphoma provide a tumor-specific Ag and a target for immunotherapy. We have developed several generations of idiotype vaccines that were tested in an animal model, the 38C13 mouse B cell lymphoma. Intitially we showed that effective tumor immunity was elicited by the syngeneic Id when it was conjugated to a carrier protein and mixed with an adjuvant. A subsequent generation of Id vaccines eliminated the need for a carrier protein and for an adjuvant by incorporating cytokines into fusion proteins containing the Id. A third generation of vaccines consisting of naked DNA encoding the Id-granulocyte-macrophage colony-stimulating factor (GM-CSF) fusion proteins was equally effective in inducing tumor immunity. To determine whether Ig variable regions, in the absence of constant regions, could be immunotherapeutic in this model, we tested the use of single-chain Fv (scFv). scFv proteins, produced in bacteria, and naked DNA encoding scFv were used in this study. scFv was tested alone or fused to GM-CSF or an immunoenhancing peptide derived from IL-1β. Here we demonstrate that scFv-GM-CSF was effective only when injected as a protein, not as a DNA vaccine. In contrast, both scFv-IL-1β peptide fusion protein and naked DNA encoding it induced tumor immunity that protected mice from tumor challenge. ***The Journal of Immunology,*** **1996, 157: 5503–5511.**

The Id expressed by the variable regions of the Ig molecule on malignant B cells provides a tumor-specific Ag and thus serves as a target for immunotherapy. In murine B cell tumor models, we and others have demonstrated that both passive (1–3) and active (4–8) immunotherapeutic treatments can cure mice with established tumors. These studies formed the basis for our idiotypic immunotherapy trials for patients with follicular lymphoma. The patients were treated by passive administration of anti-idiotypic Ab (9–12) or by active vaccination with an immunogenic formulation of Ig derived from their own tumor (13–15). Both approaches were shown to be beneficial. The advantage of the active approach is that it induces a polyclonal anti-Id response that may prevent the emergence of tumor cells with mutations in their Ig V regions (16, 17). The added advantage of the active vaccination approach is that the immunogen is administered directly to the patients without the need to produce anti-Id mAbs in rodents, which makes this treatment more economical and more timely.

To design the most effective B cell tumor Id vaccine, we assayed several generations of vaccines in a murine model system. Originally, using protein vaccines, we found that the syngeneic Id needed to be conjugated to a strong carrier such as KLH and to be mixed with an adjuvant to induce a tumor-protective anti-idiotypic response (4). Later, we demonstrated that a soluble "built in" adjuvant could be substituted for a carrier protein and an adjuvant (18, 19). Soluble fusion proteins composed of Id genetically coupled to cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF),[3] IL-2, IL-4, and IFN-γ, enhanced the immunogenicity of the Id and elicited an anti-Id response that protected mice from tumor challenge (18, 19). These Id-cytokine fusion proteins contained chimeric Ig made of murine variable regions and human constant regions. It is conceivable that the foreign human constant regions may have played a carrier role that augmented the immune response against the syngeneic Id. Since the anti-Id vaccination strategy is ultimately intended for use in humans, it was deemed important to eliminate the foreign constant regions and produce a completely syngeneic Id-cytokine.

The approach taken here was to construct single-chain Fv (scFv) containing the 38C13 V_H and V_L linked by a flexible peptide spacer $(Gly4Ser)_3$. scFv is the smallest Ig fragment that retains a complete Ag binding site (20–25). In many instances the affinity of scFv for its Ag has been shown to be similar to that of the native Ab from which it was derived (20, 26–29). In the current study, we prepared scFv alone or fused to cytokines. We reasoned that if scFv proteins prove to be effective tumor vaccines, then variable region genes could be rapidly isolated by PCR from tumor samples and expressed as scFv in bacteria. For the cytokines, we chose GM-CSF, the most effective of several cytokines tested in our previous studies, as well as a peptide sequence derived from IL-1β. This IL-1β peptide has been previously shown to maintain the immunostimulatory effect of the whole cytokine and to avoid the harmful pyrogenic action of IL-1β (30–33). The nonapeptide sequence (IL-1β peptide), VQGEESNDK, is derived from amino acids 163–171 of the cytokine and corresponds to an amphipathic region that is crucial for binding IL-$1R_I$ (34). IL-1β peptide was shown previously to be effective in T cell-dependent and T cell-independent immune responses and to enhance both primary and secondary responses (35).

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[3] Abbreviations used in this paper: GM-CSF, granulocyte-macrophage colony-stimulating factor; scFv, single-chain Fv; IL-1β peptide, amino acids 163–171 of IL-1β; mGM-CSF, mouse GM-CSF; HRP, horseradish peroxidase; KLH, keyhole limpet hemocyanin; SAF, Syntex adjuvant formulation.

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A second approach was to eliminate altogether the need to produce scFv proteins in bacteria and instead immunize mice with scFv naked DNA vaccines. DNA vaccination has been proven effective in eliciting humoral and cytotoxic immune responses (36–38). The vaccine consists of a mammalian expression vector containing a gene of interest and is injected into the muscle, where protein is produced and responded to by the immune system (39–42). DNA encoding Ig variable-region genes has been used as a vaccine (43–45) and has been shown to evoke anti-Id immune responses in the BCL-1 tumor model (3, 45). In the 38C13 B cell tumor model, we recently demonstrated that mice immunized with plasmids encoding chimeric Id (38C13 variable regions and human constant regions) or chimeric Id-GM-CSF were protected from tumor challenge (46). To test the potential of scFvs as DNA vaccines, we constructed a eukaryotic expression plasmid encoding scFv alone or fused to GM-CSF or IL-1β peptide. Of these scFv DNA vaccines, only those containing the IL-1β peptide sequence induced an anti-Id response that protected mice from tumor challenge. These results differed from those obtained with vaccination with proteins as immunogens, where both GM-CSF and IL-1β peptide scFv fusion proteins elicited a protective anti-Id response.

Materials and Methods

Mice and cell lines

Six- to nine-week-old female C3H/Hen mice were obtained from Simonsen Laboratories (Gilroy, CA) or from Charles River Breeding Laboratories (Raleigh, NC) and housed at the Laboratory Animal Facility at Stanford University Medical Center (Stanford, CA).

38C13 is a carcinogen-induced B cell tumor (47). 38C13/V2 is a variant that does not express cell-surface immunoglobulin (48). A rescue hybridoma secreting 38C13 IgM (38C13/A1-2) and hybridomas secreting anti-Id Ab S1C5, S5A8, S3H5, and S4C8 have been described previously (1). Cell lines were maintained in RPMI 1640 (Life Technologies, Grand Island, NY), 10% FCS, and 50 μM 2-ME at 37°C and 5% CO_2 in a humidified incubator. The hybridoma 9E10.2 was obtained from the American Type Culture Collection (Rockville, MD), grown as ascites in mice, and used to detect the c-*myc* peptide tag (49). COS 7.0 cells were grown in DMEM and 10% FCS at 37°C and 5% CO_2 in a humidified incubator.

PCR amplification

Reactions were performed in a final volume of 50 μl containing 1 μM of each primer, 250 μM of each dNTPs and 5 U of Taq DNA polymerase (Bethesda Research Laboratories, Gaithersburg, MD) or Pfu I DNA polymerase (Stratagene, La Jolla, CA). Amplification consisted of an initial denaturation step of 5 min at 94°C, followed by 35 cycles each at 94°C for 1 min, 55°C for 50 s, and 72°C for 90 s, with a final extension step of 5 min at 72°C, using a Perkin Elmer thermal cycler (Norwalk, CT). The following primers for PCR amplification were synthesized by Operon (Emeryville, CA) or by the protein and nucleic acid facility (Beckman Center for Molecular and Genetic Medicine, Stanford, CA):

IL-1β-F: 5′-GGCCGCAGTTCAGGGTGAAGAAAGTAACGATAAAGC
IL-1β-R: 5′-GGCCGCTTTATCGTTACTTTCTTCACCCTGAACTGC
sIL-1β-F: 5′-GGCCGCAGAAGGTAACGAAAAAGATCAGGTTAGTGC
sIL-1β-R: 5′-GGCCGCACTAACCTGATCTTTTTCGTTACCTTCTGC
mGM-CSF-F: 5′-ATGCGGCCGCAGGCGGAGCACCCACCCGC
mGM-CSF-R: 5′-TCTGCGGCCGCGCGTTTTTGGACTGGTTT
scFv-F: 5′-GGTGCACGATGTGAGGTGAAGCTG
scFv-R: 5′-CGGGATCCCGGGAATTCTTATTAATTCAG

Construction of scFv plasmids

All scFvs were derived from the Ig of the 38C13 B cell tumor and inserted into a bacterial expression vector or into a mammalian expression plasmid.

Bacterial expression plasmids: pscFv. The Ig variable-region genes were PCR amplified from 38C13 cDNA using forward and backward primers homologous to the published sequences (50). After gel purification the V_H and V_K products were mixed with complementary oligonucleotides that also code for a flexible peptide $(Gly_4\ Ser)_3$ and were amplified with *Sfi*I-VH38C-F and *Not*I-VK-38C-R primers. The products were digested with *Sfi*I and *Not*I and cloned into a modified pUC119 vector that contains sequences coding for the bacterial leader pelB (51) and the human c-*myc* peptide tag. This plasmid was constructed by Dr. Nigel Low in the laboratory of Dr. Greg Winter, Medical Research Council, Cambridge, U.K.

Bacterial expression plasmids: pscFv-mouse GM-CSF. The mouse (m)GM-CSF gene was amplified by PCR from the plasmid SV20-38C/mGM-CSF (18) using specific primers incorporating *Not*I sites and inserted into pscFv.

Bacterial expression plasmids: pscFv-IL-1β peptide. The nonapeptide VQGEESNDK is derived from amino acids 163–171 of the human IL-1β and was cloned into the *Not*I site of pscFv by two complementary and overlapping (IL-1β-F and IL-1β-R) phosphorylated oligonucleotides encoding the peptide and incorporating a *Not*I site. A control plasmid containing the scrambled sequence EGNEKDQVS (sIL-1β peptide) was similarly constructed using sIL-1β-F and sIL-1β-R oligonucleotides.

Eukaryotic expression plasmids. The scFv regions were PCR amplified from the bacterial expression plasmids using primers that incorporate upstream *Dra*III and downstream *Bam*HI sites. scFv genes were inserted into a mammalian expression plasmid (46, 52) driven by the CMV promoter, downstream of the human κ leader sequence and upstream of a SV40 polyadenylation site. Production of the proper proteins was tested by transfecting COS 7.0 cells and analyzing the secreted proteins by ELISA as described below and by the ability of the scFv-mGM-CSF to support the growth of the GM-CSF-dependent cell line, NFS-60. For injection, closed circular plasmid DNA was isolated using the Wizard Megaprep Purification Kit (Promega Corp., Madison, WI). Purified DNA was reprecipitated, washed twice with 70% ethanol, and resuspended in saline. The ratio of $OD_{260/280}$ ranged from 1.8 to 2.0.

Expression and purification of scFv proteins

Plasmids expressing the various scFv in *Escherichia coli* (Sure strain) were grown overnight in Super Broth medium containing 0.1% glucose and 100 μg/ml carbenicillin at 37°C. Expression of scFv proteins was induced by the addition of 1 mM isopropyl thiogalactose, followed by incubation for 16 to 24 h at 25°C. The bacteria were centrifuged at 4,000 [times] *g* for 20 min at 4°C and resuspended in 50 ml of PBS containing protease inhibitors (0.5 ml of a 100 [times] solution containing 0.0028% leupeptin, 0.014% pepstatin A, 1.7% PMSF, and 3.3% benzamidine in ethanol). To extract proteins from the periplasmic space, bacteria were lysed by lysosyme (1 mg/ml) and kept on ice for 30 min, followed by sonication and centrifugation at 10,000 [times] *g* for 10 min at 4°C. The pellet was kept on ice and resuspended in PBS containing 0.01% Nonidet P-40, sonicated again, and centrifuged (10,000 [times] *g* for 10 min at 4°C). The soluble periplasmic proteins in the supernatant were filtered (0.45-μm Millipore® filter) and applied to an anti-Id affinity column containing Sepharose conjugated with the S1C5 mAb (1). Bound scFv proteins were eluted by 50 mM of diethanolamine, pH 12, and dialyzed against PBS (53). The yield of the purified scFv fusion proteins, obtained from the different constructs, varied from 1.5 to 2.0 mg/L. Endotoxin assays, based on Limulus amebocyte lysate clot assays, were performed and were below 1 endotoxin U/μg of protein.

Immunization with scFv proteins and with plasmid DNA encoding scFv followed by tumor challenge

Soluble scFv proteins (15 μg) were injected i.p. into mice three times at 2-wk intervals in a total volume of 0.25 ml of PBS. Sera were collected by tail-vein bleeding 10 days after each vaccination. Two weeks after the last injection, mice were injected i.p. with 200 viable 38C13 tumor cells. Plasmid DNA (100 μg) was injected three times at 3-wk intervals in both hind thigh muscles in a total volume of 100 μl of saline. Seventeen days after the second and the third injections, immune sera were collected by tail vein bleeding. Three weeks after the last injection, mice were injected i.p. with 200 viable 38C13 tumor cells. Statistical comparison of survival were done using the log rank test.

Immunoassays

Reactivity and secondary structure analysis of scFv proteins were determined by ELISA. Microtiter plates (Nunc, Naperville, IL) were coated overnight at 4°C with anti-idiotypic mAbs (1) as indicated in the individual experiments, at a concentration of 5 μg/ml in carbonate buffer (50 mM $NaHCO_3$, pH 8.0). Residual binding sites were blocked with 5% w/v nonfat milk in PBS for 20 min. Plates were washed with 0.15 M NaCl containing 0.05% Triton X-100, and serially diluted scFv proteins were applied in a total volume of 50 μl in 2% BSA/PBS and incubated for 1 h at room temperature. Plates were washed before the addition of 50 μl (2.5 μg/ml)

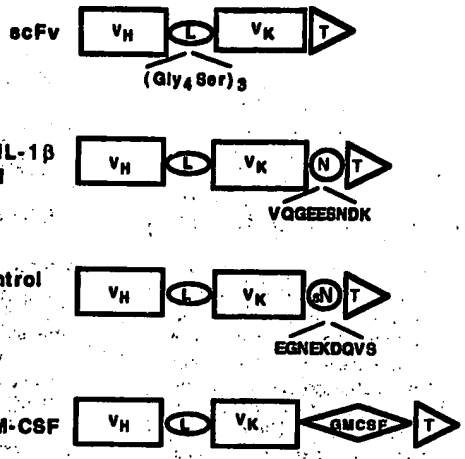

FIGURE 1. Schematic representation of 38C13 scFv proteins. The V_H and the V_L are linked by a flexible linker $(Gly_4Ser)_3$, and "T" marks the location of a c-*myc* peptide tag fused at the carboxyl-terminal end. Mouse GM-CSF, the IL-1β nonapeptide (N), as well as a scrambled control sIL-1β nonapeptide (sN) were inserted upstream of the tag peptide.

of an anti-c-*myc* mAb, 9E10 (IgG1), or anti-mGM-CSF (Genzyme, Cambridge, MA) and were incubated for 1 h at room temperature. Plates were washed, and 50 μl of the appropriate detector, either goat anti-mIgG-HRP (Southern Biotechnology Associates, Birmingham, AL) or goat anti-rat IgG-HRP (Tago, Burlingame, CA), was added, incubated for 1 h at room temperature and washed before adding 100 μl of ABTS [2,2'-azinobis(3-ethylbenzthiazoline sulfonic acid)] substrate (Boehringer Mannheim, Indianapolis, IN). The absorption at 405 to 450 nm was measured in a V_{max} microplate reader (Molecular Devices, Menlo Park, CA).

Anti-Id response was measured by titering sera on a microtiter plate coated with native 38C13 IgM (5 μg/ml). Bound Abs were detected using goat anti-mIgG-HRP (Southern Biotechnology Associates' A mixture of purified monoclonal anti-38C13 idiotypic Ab containing IgG1, IgG2a, and IgG2b subclasses at a ratio of 2:1:1 was used as a standard for quantitation (1).

Anticytokine responses were measured by titering sera on microtiter plates coated either with rmGM-CSF (Genzyme), IL-1β (human IL-1β and anti-IL-1β were kindly provided by Dr. Craig Reynolds, NCI, Fredrick, MD), or IL-1β peptide; bound Abs were detected using goat anti-mIgG-HRP (Southern Biotechnology Associates). Purified mAbs against mGM-CSF or IL-1β were used as standards.

SDS-PAGE and Western blotting

Proteins were separated by 12% gels and stained with Coomassie brilliant blue or transferred onto nitrocellulose membranes (Schleicher and Schull, Keene, NH) by semidry electroblotting (JKA Biotech, Denmark). The membranes were probed with the anti-Id mAb S1C5 (1), with anti-c-*myc* 9E10 (49) or with anti-mGM-CSF. The bound mAbs were incubated with the appropriate HRP-conjugated goat reagents and detected by enhanced chemiluminescence (ECL) (Amershem, Arlington Heights, IL) according to the manufacturer's directions.

Biologic activity of GM-CSF fusion proteins

scFv-fusion proteins were assayed for their ability to support the proliferation of a mGM-CSF-dependent cell line, NFS-60. Cells (10^4/well) were grown in triplicate in 96-well plates containing serially diluted purified scFv-GM-CSF-fusion proteins for 3 days using purified rmGM-CSF (Genzyme) as a standard. Cell proliferation was measured by the XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino)carbonyl]2H-tetrazolium hydroxide] assay (54). Fifty microliters of 1 μg/ml XTT (Sigma Chemical Co., St. Louis, MO) and 7 μg/ml of menadione in RPMI 1640 were added to each well and incubated for 3 to 4 h at 37°C, and the absorption at 450 nm was measured in the microplate reader.

Flow cytometry

38C13 and the surface-Ig^{neg} variant 38C13/V2 cells (5 [times] 10^5) were reacted at 4°C with 50 μl of Id-reactive serum (diluted 1:5 in 2% BSA/PBS

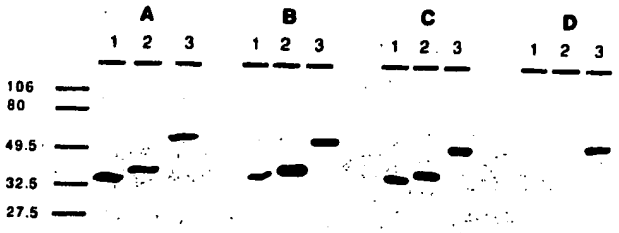

FIGURE 2. SDS-PAGE and Western analysis of scFv fusion proteins: *lane 1*, scFv; *lane 2*, scFv-IL-1β peptide; and *lane 3*, scFv-mGM-CSF. Purified scFv proteins were electrophoresed in a nonreducing 12% polyacrylamide gel and visualized by Coomassie brilliant blue staining (*A*). Duplicate gels, transferred to nitrocellulose membranes, were probed with anti-c-*myc* peptide mAb (*B*), with anti-Id mAb S1C5 (*C*), or with rat anti-mGM-CSF Abs (*D*).

containing 0.02% sodium azide). Binding of Ab to the cells was detected with goat anti-mIgG-FITC Ab (Tago). Cells fixed in 2% paraformaldehyde/PBS were analyzed with a FACScan (Becton Dickinson, Mountain View, CA).

Results

Construction of scFv proteins as Id vaccines

We tested the feasibility of using scFv proteins made in bacteria as Id vaccines. V_H and V_K from the 38C13 B cell tumor were amplified by PCR and linked by a 15-residue flexible linker $(Ser_4Gly)_3$ as detailed in *Materials and Methods*. The scFv was cloned downstream of the bacterial pectate lyase gene leader sequence (*pelB*) and tagged by the c-*myc* peptide, as illustrated in Figure 1. Preliminary experiments indicated that the soluble scFv protein was a poor immunogen. To enhance the immunogenicity of the protein, it was molecularly fused with mGM-CSF (Fig. 1). This cytokine was previously shown to be an effective immune enhancer for whole Ig proteins (18, 19).

As an alternative to GM-CSF we chose an immunoenhancing peptide that had been described to be a potent immunoenhancer when molecularly fused to Ags (31). This peptide consists of nine amino acids, VQGEESNDK (IL-1β peptide), corresponding to amino acids 163–171 of the IL-1β molecule (31, 32). It has potent immunostimulatory properties but few of the other activities of IL-1β (31, 33, 55, 56). To test the potential use of IL-1β peptide as a "built in" adjuvant for scFv, a plasmid was constructed with this sequence downstream of the scFv. A second control plasmid contained the scrambled sequence EGNEKDQVS (sIL-1β peptide) (Fig. 1).

Purification of scFv proteins

scFv proteins were produced in bacteria, and purified proteins of the expected molecular weights were detected by Coomassie brilliant blue staining of SDS-PAGE run under nonreducing conditions (Fig. 2*A*) and by Western blot analysis. The denatured proteins reacted with the anti-c-*myc* peptide Ab, 9E10 (Fig. 2*B*), the anti-Id, S1C5 (Fig. 2*C*), and, in the case of the scFv-mGM-CSF-fusion protein, with a rat anti-mGM-CSF (Fig. 2*D*).

Secondary structure of scFv proteins

To test whether the purified proteins were properly folded, we used a panel of rat and murine anti-Id mAb. These mAbs were previously produced in our laboratory and react with closely spaced epitopes, containing both V_H and V_K residues, on the 38C13 Ig molecule (1). The mAbs were used individually to capture the scFv fusion proteins on a microtiter plate, and the binding was detected with biotin-conjugated anti c-*myc* peptide or anti-mGM-CSF Ab. The native 38C13 IgM molecule was bound, as expected, to all the

Table I. *Id proteins and DNA vaccines*

Vaccine Name	Type	Expression System	Vaccine Description	Reference
scFv	Protein	Bacteria	scFv of 38C13 tumor Id	Current work
scFv-IL-1β peptide	Protein	Bacteria	scFv of 38C13 tumor Id fused to IL-1β peptide	Current work
scFv-mGM-CSF	Protein	Bacteria	scFv of 38C13 tumor Id fused to mGM-CSF	Current work
scFv-control peptide	Protein	Bacteria	scFv of 38C13 tumor Id fused to scrambled IL-1β peptide	Current work
38C13/KLH-SAF	Protein	Hybridoma	38C13 tumor Id, IgM coupled to KLH and mixed with SAF adjuvant	18, 19
TF 38C13-mGM-CSF	Protein	Transfectoma	Chimeric 38C13 tumor Id fused to mGM-CSF (human constant regions; γ1 and κ fused to mouse variable regions)	18, 19
pscFv	DNA	Eukaryotic	Plasmid DNA encoding scFv of 38C13 tumor Id	Current work
pscFv-IL-1β peptide	DNA	Eukaryotic	Plasmid DNA encoding scFv of 38C13 tumor Id-IL-1β peptide fusion protein	Current work
pscFv-mGM-CSF	DNA	Eukaryotic	Plasmid DNA encoding scFv of 38C13 tumor Id mGM-CSF fusion protein	Current work
p38C13	DNA	Eukaryotic	Plasmid DNA encoding chimeric 38C13 tumor Id (human constant regions; γ1 and κ fused to mouse variable regions)	46
p38C13-mGM-CSF	DNA	Eukaryotic	Plasmid DNA encoding chimeric 38C13 tumor Id-mGM-CSF fusion protein (human constant regions; γ1 and κ fused to mouse variable regions)	46
pBCL1	DNA	Eukaryotic	Plasmid DNA encoding chimeric BCL1 tumor Id (human constant regions; γ1 and κ fused to mouse variable regions)	46

anti-idiotypic Abs and detected by an anti-IgM Ab. An identical binding pattern was seen with the scFv-IL-1β peptide fusion protein, indicating that the V_H/V_K combined epitope was recreated and that the molecule was properly folded. scFv-mGM-CSF was reactive with the anti-idiotypic and with anti-mGM-CSF mAb. The scFv-mGM-CSF molecule supported the growth of the GM-CSF-dependent cell line, NFS-60. However, the GM-CSF activity was lower than either the monomeric rGM-CSF or the dimeric 38C13-GM-CSF (data not shown). It is possible that the cytokine domain was less active because of its molecular location, between the scFv and the peptide tag.

Immunogenicity of scFv fusion proteins

Mice were immunized with the various forms of the scFv fusion proteins, and their humoral responses were compared with those of mice that were injected with 38C13 IgM coupled to KLH and mixed with the SAF adjuvant (4) or to mice that were injected with the soluble transfectoma-derived TF-38C13-mGM-CSF (18, 19) (Table I). C3H/Hen mice were injected i.p. with equimolar concentrations of Id proteins, 15 μg of soluble scFv, or 50 μg of 38C13 IgM as detailed in *Materials and Methods*, and serum was collected at 2-wk intervals. The anti-idiotypic immune response was determined by ELISA on plates coated with the native 38C13 IgM protein and detected with goat anti-mIgG Ab. After the primary immunization, scFv-immunized mice had no detectable anti-idiotypic IgG Ab. In contrast, mice that were immunized with 38C13-KLH/SAF and those immunized with the soluble TF-38C13-mGM-CSF, showed low titers of anti-idiotypic IgG Ab (data not shown) that were boosted after the second immunization (day 24) (Fig. 3). Mice that were immunized with scFv fused to either IL-1β peptide or mGM-CSF showed a low but reproducible humoral response after the first boost (day 24) that was further elevated by an additional immunization (day 40). The soluble scFv protein coupled to KLH and the control scFv fused to the scrambled IL-1β peptide sequence (scFv-control peptide) did not elicit an anti-idiotypic response even after repeated injections (data not shown). The subclass content of the anti-idiotypic Ab induced in responding mice differed in these groups. While TF-mGM-CSF protein induced mostly IgG1 Ab, scFv proteins elicited both IgG1 and IgG2a Ab, in ratios that were similar to those induced by the native 38C13-KLH immunogen (Table II).

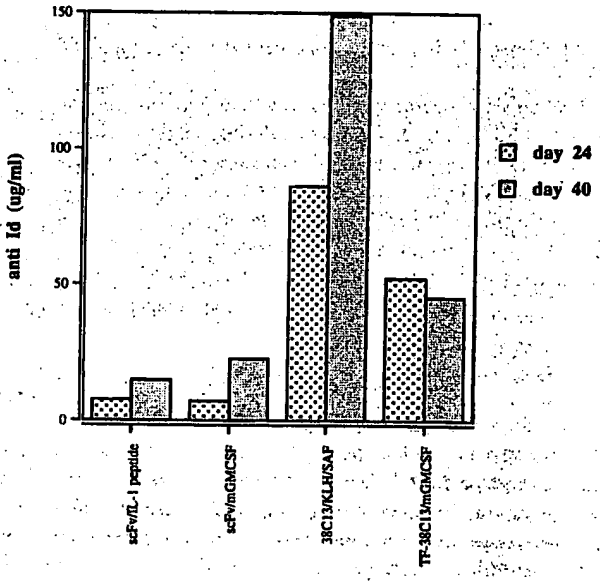

FIGURE 3. Anti-Id titers in protein-immunized mice. Mice were injected i.p. with 15 μg of soluble scFv proteins, with 50 μg of soluble transfectoma-derived 38C13-mGM-CSF or with 50 μg of the native IgM (38C13) that was coupled to KLH and mixed with adjuvant. Sera were collected 10 days after each immunization and analyzed by ELISA. Results are shown for pooled sera 10 days after the second immunization (day 24) and 10 days after the third immunization (day 40). The results are representative of four experiments.

scFv fusion proteins elicit Ab that react with 38C13 tumor cells

The immune sera were tested for binding to the 38C13 tumor cells and to an Ig^{neg} variant, 38C13/V2 (48). All mice that responded to the scFv fusion proteins produced Ab that bound the parental 38C13 tumor cells (Fig. 4*A*) but not the Ig^{neg} variant (Fig. 4*B*). These results indicate that the soluble scFv-fusion proteins induce anti-idiotypic Ab that recognize specifically the native IgM on the cell surface (Fig. 4).

Table II. *Analysis of Ig subclasses in sera of mice responding to Id vaccines*

Protein Vaccination[a]			DNA Vaccination[b]		
Vaccine	IgG2a/IgG1	Total IgG (μg/ml)	Vaccine	IgG2a/IgG1	Total IgG (μg/ml)
scFv		0	pscFv	>40	0.58
scFv-IL-1β peptide	0.27	14.8	pscFv-IL-1β peptide	9.5	1.15
scFv-mGM-CSF	0.42	21.2	pscFv-mGM-CSF		0
38C13/KLH-SAF	0.32	149	p38C13	13	1.38
TF 38C13-mGM-CSF	<0.001	104	p38C13-mGM-CSF	0.6	1.52

[a] Mice were injected three times at 2-wk intervals, and serum was analyzed 10 days after the third injection. Sera of responding mice were pooled and assayed by ELISA.

[b] Mice were injected three times at three-wk intervals, and serum was analyzed 17 days after the third injection. Sera of responding mice were pooled and assayed by ELISA.

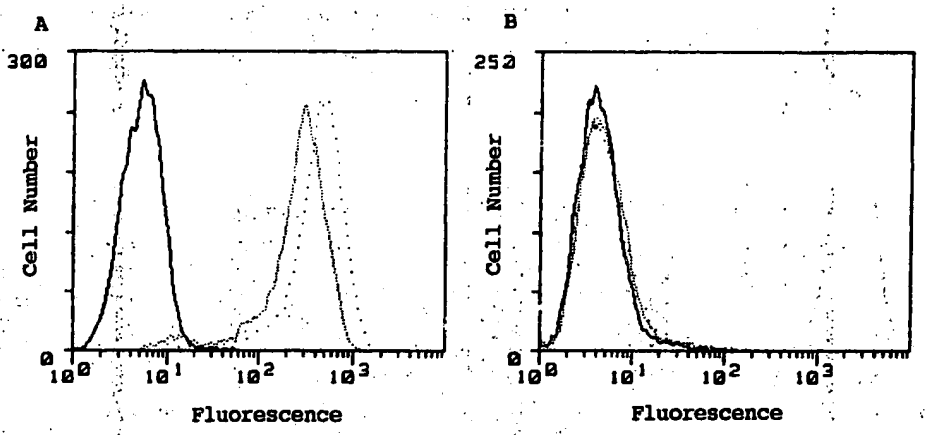

FIGURE 4. Anti-idiotypic Abs elicited by scFv fusion proteins bind to 38C13 cells: flow cytometry analysis of sera from mice immunized three times with scFv-IL-1β peptide. Immune sera were reacted with 38C13 cells (*A*) or V2 (*B*), an Ig-negative variant of 38C13, and detected with goat anti-mouse IgG-FITC Abs. The solid line (—) indicates the fluorescence of cells stained with normal serum followed by the second step Abs, and the dotted lines indicate the fluorescence of cells stained with serum from mice immunized with scFv-IL-1β peptide (----) and cells stained with an anti-Id mAb S1C5 (· · ·).

Analysis of anti-GM-CSF and anti-IL-1β response in mice immunized with the corresponding cytokine fusion proteins

We previously reported that repeated immunization with TF-38C13-mGM-CSF-fusion protein induces an anti-GM-CSF autoantibody response (57). Since mGM-CSF is dimeric in the TF-38C13-mGM-CSF but monomeric in the scFv protein, we wished to determine whether Abs against mGM-CSF were induced in mice immunized with scFv-mGM-CSF. Sera from mice immunized with scFv-mGM-CSF were serially diluted on ELISA plates coated with mGM-CSF, and bound Ab were detected with goat anti-mIgG. This analysis showed that although the monomeric cytokine in scFv-mGM-CSF elicited anti-GM-CSF Ab, it was not as immunogenic as the dimeric cytokine molecule, TF-38C13-mGM-CSF (data not shown).

Similarly, we tested whether Abs against IL-1β or the IL-1β peptide were induced in mice immunized with scFv-IL-1β peptide. ELISA plates were coated with either human IL-1β or with the IL-1β peptide, sera from mice immunized three times with scFv-IL-1β peptide were serially diluted, and bound Abs were detected with goat anti-mIgG. No humoral immune response against either the human IL-1β r the IL-1β peptide was detected (data not shown). Moreover, the hyperimmune serum was not reactive with an valbumin-IL-1β peptide fusion protein when tested by Western analysis (H. Maecker and S. Levy, unpublished results).

Immunogenicity of DNA encoding scFv

We have shown that DNA coding for the TF-38C13 can induce an anti-idiotypic immune response even though the TF-38C13 protein is unable to do so without the addition of mGM-CSF (46). Therefore, it was of interest to compare the immunogenicity of scFv DNA with TF-38C13. Mice were injected with DNA encoding scFv or with DNA encoding scFv-fusion proteins and compared with mice immunized with DNA encoding chimeric TF-38C13. Mice were injected with 100 μg of each plasmid using two routes of injection, i.m. and i.d. Also, two different schedules of injections, three times weekly or three times every 3 weeks were tested for the i.m. route. The results indicate that the immunization schedule was critical for this Ag system. No humoral immune responses were detected in mice injected i.m. three times on the weekly schedule, whereas mice injected every 3 wk made immune responses (Table II).

We recently showed that DNA vaccination with p38C13 or p38C13-mGM-CSF induced an anti-Id response (46). Surprisingly, of the DNA encoding scFv constructs, the scFv-mGM-CSF was not immunogenic even after the third injection (Table III), yet mice injected with plasmid DNA encoding either scFv r scFv-IL-1β peptide showed detectable low titers of anti-Id IgG after two injections. However, the percentage f responding mice was variable. After the third injection most of the mice in the two responding groups made an

Table III. *Anti-Id response to scFv DNA vaccines*

	scFv		scFv-IL-1β Peptide		scFv-mGM-CSF	
	Responding mice (%)	Anti-Id (μg/ml)	Responding mice (%)	Anti-Id (μg/ml)	Responding mice (%)	Anti-Id (μg/ml)
Second immunization	80	0.35	50	0.34	0	0
Third immunization	100	0.4	80	0.7	0	0

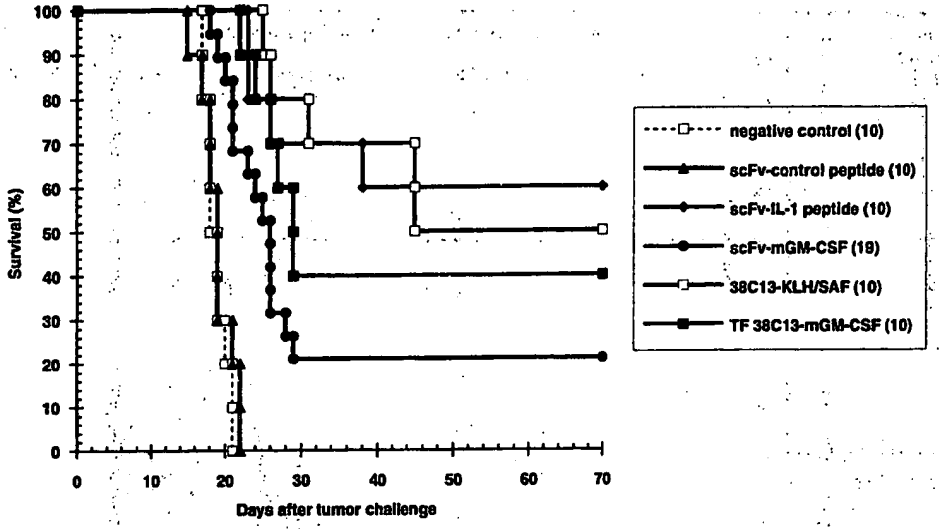

FIGURE 5. Survival of mice immunized with scFv proteins and challenged with tumor. C3H/Hen mice (10–19 per group, as indicated) were immunized three times at 2-wk intervals with scFv-IL-1β peptide, scFv-control peptide, and scFv-mGM-CSF. Controls included mice that were immunized with 38C13-KLH/SAF or 38C13-GM-CSF and nonimmunized mice. Two weeks after the last immunization, mice were challenged with 200 38C13 tumor cells and followed for survival. The results are representative of three experiments.

anti-Id immune response that was boosted only in mice injected with plasmid DNA encoding scFv-IL-1β peptide (Table III). The ratio of IgG2a to IgG1 in the responding mice was consistently higher in the DNA-vaccinated mice than in the protein-vaccinated mice, but the total IgG produced was much higher in mice immunized with protein (Table II).

Tumor protection of mice immunized with scFv cytokine fusion proteins

Previously, we demonstrated that mice immunized with the proteins TF-38C13-mGM-CSF and with 38C13-KLH/SAF were protected from tumor challenge (18, 19). Therefore, the fusion proteins containing the IL-1β peptide or the mGM-CSF were similarly tested for their ability to induce resistance to the tumor. Mice were challenged with 200 38C13 tumor cells 2 wk after the last immunization (Fig. 5). Nonimmunized mice died of tumor by day 22 with a mean survival of 19 days. The survival of mice immunized with the control scrambled IL-1β peptide was nearly identical to that of the nonimmunized mice. In contrast, mice that were immunized with the positive controls, soluble TF-38C13-GM-CSF, or 38C13-KLH/SAF were protected against tumor challenge, with 44 and 50% of long-term survivors and 52- and 62-day mean survival, respectively ($p < 0.0001$). Mice that were immunized with scFv-mGM-CSF were less protected against tumor challenge, with only 20% long-term survivors and a mean survival of 32 days ($p = 0.0003$). Surprisingly, the IL-1β peptide fusion proteins, which did not elicit a strong Ab response, protected animals against tumor challenge. These mice immunized with scFv-38C13-IL-1β peptide had 60% long-term survivors with a mean survival of 65 days ($p < 0.0001$). These values are not significantly different from the survival of mice in the two positive control groups ($p = 0.85$ and $p = 0.42$, respectively). In all protected groups there was no correlation between the level of Ab in individual mice and their degree of tumor protection. This lack of correlation has been previously noted for whole-protein vaccination in this system (8, 58).

Tumor protection of mice immunized with DNA

To determine whether DNA vaccination could protect mice from tumor challenge, mice were injected three times at 3-wk intervals with 100 μg of plasmids encoding various forms of scFv and scFv cytokine fusion proteins (Table I). A positive control group of mice was injected with p38C13 (46), and a negative control group of mice was injected with DNA encoding an irrelevant Id (BCL1). Three weeks after the last injection, mice were challenged with 200 38C13 tumor cells i.p. The survival data are shown in Figure 6. Mice injected with plasmid encoding BCL1 or scFv-GMCSF died of tumor by day 22 with a mean survival of 19 days. Mice that were immunized with scFv plasmid DNA died by day 32 with a mean survival of 21 days. Mice that were injected with plasmids encoding chimeric 38C13, the positive group, and with scFv-IL-1β peptide were protected against tumor challenge, with 40 and 30% of long-term survival and 40- and 37-day mean survival, respectively ($p = 0.011$ and $p = 0.012$, respectively).

Discussion

These studies are an extension of our earlier work, in which we used the murine B cell tumor 38C13 model system to test possible approaches for the treatment of human B cell lymphomas. We

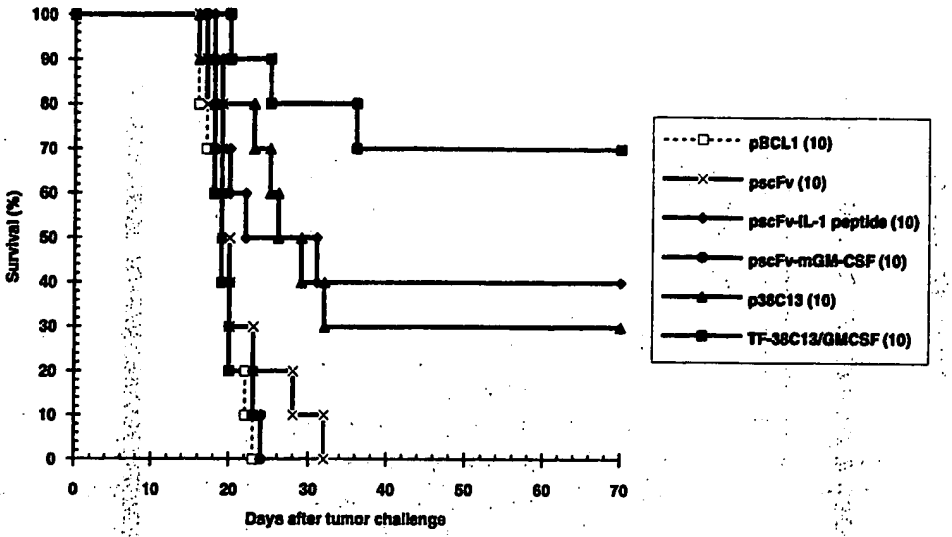

FIGURE 6. Survival of mice immunized with DNA and challenged with tumor. C3H/Hen mice (10 per group) were immunized three times at 3-wk intervals with pscFv, pscFv-IL-1β peptide, and pscFv-mGM-CSF. Controls included mice that were immunized with DNA, p38C13, and with protein, TF-38C13-mGM-CSF. Three weeks after the last immunization, mice were challenged with 200 38C13 tumor cells and followed for survival. The results are representative of two experiments.

previously demonstrated that the 38C13-Id can be used as a tumor-specific Ag and that both passive (1) and active (4, 6–8) immunotherapy could protect animals from tumor challenge. In these experiments, anti-idiotypic Abs alone, above a certain threshold, were required for tumor protection (8, 58). Active vaccination was dependent on the use of immunoenhancing modalities that acted to elicit an anti-idiotypic response in the syngeneic animals. Thus, while the 38C13IgM molecule alone was nonimmunogenic, it could be made immunogenic by coupling it to KLH and then mixing the coupled protein with an adjuvant (1). Others have reported that some idiotypes injected into mice, in the presence of adjuvants, can induce anti-idiotypic immune responses in the absence of a carrier protein (59). However, for human vaccination there is a need to increase the immunogenicity of the B cell lymphoma Id. For these reasons we have been seeking approaches aimed at increasing the immunogenicity of soluble Id using the murine model system.

One approach that we recently explored is the use of cytokines fused to the Id as "built-in" immunoenhancers (18, 19). We first used GM-CSF and genetically fused it to a whole Ig molecule, showing that the soluble protein was indeed immunogenic and that the anti-idiotypic immune response protected animals from subsequent tumor challenge (18). A fusion protein made with a mouse inactive cytokine, human GM-CSF, was ineffective. Subsequently we tested soluble Id-cytokine fusion proteins containing either IL-2 or IL-4 and demonstrated that these cytokines were also able to enhance the immune response to the Id and that this response protected animals challenged with the 38C13 tumor (19).

Our current studies were aimed at analyzing the potential use of scFvs as immunogens for B cell lymphomas. scFv can be easily obtained from tumor samples by gene amplification (45) and cloned and expressed in bacteria (20). The use of bacteria for protein expression, rather than eukaryotic protein expressi n systems, could potentially expedite vaccine preparati n. scFv proteins have been demonstrated by others t form a three-dimensi nal structure that is similar to the original Ig from which they were derived, as analyzed by binding t an Ag (20, 27, 29) r to anti-idiotypic Ab (60). We similarly tested the 38C13-derived scFvs by their ability to bind a panel of anti-idiotypic Abs, as there is no Ag known to bind the tumor Ig. The scFv proteins bound four anti-idiotypic mAbs, as did the native 38C13 IgM, indicating that the scFv domain of the molecule was properly folded. However, scFv-GM-CSF fusion proteins, while accessible to the anti-idiotypic and to anti-GM-CSF mAbs, were not equally detected in their native state by the anti-c-*myc* tag Ab. The c-*myc* was clearly expressed and detected in the SDS-denatured molecule (Fig. 2).

The best evidence that the scFv proteins were properly folded and behaved similarly to the native Id comes from their ability to induce, in vivo, anti-idiotypic Abs that bind the 38C13 tumor cells. The anti-idiotypic Abs elicited by the scFv fusion proteins bound only the parental 38C13 tumor and not an Ig^{neg} variant (Fig. 4), nor an irrelevant murine B cell lymphoma (BCL1) (data not shown).

The scFv proteins also behaved similarly to the native IgM molecule in that the protein alone was not immunogenic. It was necessary to fuse an immunoenhancing domain to the scFv protein to invoke an immune response in mice. In these experiments the "built-in" adjuvant was a complete GM-CSF molecule, the most effective cytokine in our previous work (18, 19). In addition, we explored the use of an immunoenhancing peptide, derived from IL-1β. In our previous experience this peptide was found to act as an adjuvant and to enhance Ab response to KLH-conjugated Id (M. J. Campbell and R. Levy, unpublished observations). The IL-1β peptide corresponds to a hydrophilic region of the cytokine and was originally shown to enhance the immunogenicity of human ferritin and salmonella flagellin in mice. In both cases the peptide was molecularlly fused to the proteins (31). The mode of action of the peptide has been explored, by the same group, in recent experiments in which the peptide was shown to be essential for binding the IL-1R_I. Replacement f this region with a hom l-gous region derived from the IL-1Ra, the receptor antag nist, completely abolished binding to IL-1R_I but not t IL-1R_{II} (34).

The GM-CSF c mponent of the fusion protein was active as it supported the growth of the GM-CSF-dependent cell line, NFS-60 (data not shown). Unlike the transfectoma-derived 38C13-GM-CSF protein that was a very potent immunogen in the primary

immune response, the immunogenicity of the scFv-GM-CSF protein was manifested only after boosting. It is interesting to note that while the TF-mGM-CSF protein induced mostly IgG1 Ab, confirming previous results from our laboratory (19), the scFv-mGM-CSF induced, in addition to IgG1, Ab of the IgG2a subclass (Table II). The differences between the transfectoma-derived and the bacterially derived proteins may be due to the fact that the eukaryotic product is in a dimeric form, is likely to be properly glycosylated, and is more active than the yeast-derived rGM-CSF (18), whereas the bacterial product is monomeric, nonglycosylated, and less active than the rGM-CSF standard. Nevertheless, mice injected with the soluble scFv-mGM-CSF made an anti-GM-CSF response (data not shown). An anti-GM-CSF response was previously seen in mice hyperimmunized with the eukaryotically produced TF-mGM-CSF fusion protein. In those experiments, we tested the hyperimmunized mice for possible harmful effects of such anti-GM-CSF Abs. The experiments revealed that the only effect seen in the presence of anti-GM-CSF Abs was the reduced immunogenicity of an independent GM-CSF-fusion protein (57). In contrast, the fusion proteins made with the IL-1β peptide did not induce an immune response against IL-1β.

Although scFv-mGM-CSF protein induced an anti-idiotypic response, the immunized mice were not consistently protected from tumor challenge. In contrast, mice that were immunized with the scFv-IL-1β peptide fusion protein were consistently better protected. Survival of mice immunized with the scFv-IL-1β peptide was very similar to that of mice immunized with regimens known to be protective against tumor challenge, namely 38C13-KLH/SAF and 38C13-GM-CSF (Fig. 5). The immunostimulatory activity of the peptide was sequence specific since the control scrambled IL-1β peptide was nonimmunogenic. Although the mechanism of action of the IL-1β peptide is not clear, we have documented its immunostimulatory effects in the context of another immunogen, OVA. Furthermore, mice injected with OVA-IL-1β peptide responded with a Th1-like immune response.[4] Earlier experiments in the 38C13 tumor model indicated that there is no direct correlation between tumor protection and the level of anti-idiotypic Ab made by the vaccinated mice. Protection from tumor challenge was attained once a threshold level of 1 μg/ml of anti-idiotypic Ab was reached (4, 8). scFv-IL-1β peptide fusion protein induced approximately 15 μg/ml of anti-idiotypic Ab, which was sufficient for tumor protection (Table II).

Interestingly, the DNA construct encoding the scFv-IL-1β peptide was effective in inducing a protective tumor immune response. This construct induced a low level of Ab, just above the threshold level needed to induce protective tumor immunity. By comparison, DNA encoding scFv induced an even lower level of anti-Id Ab, below the threshold, and did not protect mice from tumor challenge. The addition of mGM-CSF did not restore immunogenecity to the scFv construct. Likewise, in other experiments (46), mGM-CSF was not able to make immunogenic the DNA encoding 38C13 variable regions in the context of mouse constant regions. Apparently, the IL-1β peptide can provide a more potent immunostimulatory effect when incorporated into a DNA vaccine than can GM-CSF.

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Acknowledgments

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